Synthetic and metabolic studies on β,γ-unsaturated α-amino acids.

By

K. O. Hallinan.

Submitted for the degree of
Doctor of Philosophy.

The University of Warwick.
Department of Chemistry.

January 1992.

CONTENTS.

Chapter Three.

Investigations into the stereochemistry of the isopenicillin N synthase catalysed cyclisation of an unnatural specifically labelled precursor.

Chapter Four.

Preparation of vinylglycine and other β,γ-unsaturated α-amino acids by the Neber rearrangement.

Chapter Five.

The development of a reagent for the determination of enantiomeric excess of chiral carboxylic acids by nmr.

Chapter Six.

Experimental details.

List of Figures.

List of tables.

ACKNOWLEDGEMENTS.

I wish to express my gratitude to Professor D. H. G. Crout for the advice, guidance, and encouragement which he extended during the course of this research at Warwick.

I would like to thank the academic staff for helpful suggestions offered and express my gratitude to the technical staff for running nmr and MS spectra.

I would also like to thank Alice Parton for her help in preparing this thesis and finally all my co-workers for making my time at Warwick most enjoyable.

The financial assistance of the Science and Engineering Research Council is gratefully acknowledged.

DECLARATION.

The work described in this thesis is the original of the author, except where acknowledgement has been made to results and ideas previously published. It was carried out at the Department of Chemistry, University of Warwick between January 1988 and July 1991 and has not been submitted previously for a degree at any institution.

SUMMARY.

(1) Chapter three describes the attempted investigation of the mechanism of cepham formation catalysed by the enzyme isopenicillin N synthase of *Acremonium cephalosporium*. The stereochemistry of this cyclisation was to be investigated with a specifically labelled butyrate residue in the unnatural substrate, δ-(α-amino)adipoyl-cysteinyl-α-aminobutyric acid. The chiral methyl and methylene groups were to be synthesised by the tritiation of specifically deuterated vinylglycine. This, in turn, was to be prepared from phenyl (2-trimethylsilylethynyl) sulphone. Scrambling of the deuterium labelling occurred in this synthesis of vinylglycine and so forced the abandonment of the experiment without concluding the investigation.

(2) Chapter four describes the preparation of vinylglycine in a simple, inexpensive, three step synthesis. The synthetic pathway involved a Pinner reaction, sodium hypochlorite treatment and an aqueous Neber rearrangement. The overall yield was 52% from the vinylcyanide. This method was applied to the production of other β,γ-unsaturated α-amino acids with mixed success. The resolution of the *t*-butyloxycarbonyl derivatives of the amino acids so prepared was investigated with the thiol protease, papain. Ethyl L-*N*-*t*-butyloxycarbonylvinylglycine prepared in this manner was transformed by epoxidation, dihydroxylation and cyclopropylation. An attempt to prepare a fluorothreonine derivative from the epoxide gave only the 2,3-dehydrohomoserine analogue.

(3) Chapter five describes the development of methyl (*R*)-(2-^{2}H)-*N*-4-methoxyphenylglycinate (I) as a potential reagent for determining enantiomeric excess in chiral acids. The diastereomers formed differ only in isotopic stereochemistry, thus the possibility of kinetic resolution is reduced to a minimum. The ee is measured by the proton nmr spectrum of the diastereomers. In coupling (I) to a range of racemic chiral carboxylic acids the expected 1:1 ratio was observed. However during one of the steps in the preparation of (I) racemisation of the proton label occurred thus a single diastereomer has yet to be prepared.

Abbreviations.

tlc	Thin layer chromatography.
HPLC	High pressure liquid chromatography.
GLC	Gas liquid chromatography.
MS	Mass spectroscopy.
IR	Infra red spectroscopy.
n m r	nuclear magnetic resonance.
p p m	parts per million.
TMS	Tetramethylsilane.
J	Coupling constant.
Hz	Hertz.
s	Singlet.
d	Doublet.
t	Triplet.
q	Quartet.
THF	Tetrahydrofuran.
DMSO	Dimethylsulphoxide.
1° residue	α-Aminoadipoyl or equivalent.
2° residue	cysteinyl or equivalent.
3° residue	valine or equivalent.
a a	α-Aminoadipoyl.
c y s	cysteinyl.
v g	vinylglycinyl.

CHAPTER 1.

ISOPENICILLIN N CYCLISATION CATALYSED BY ISOPENICILLIN N SYNTHASE.

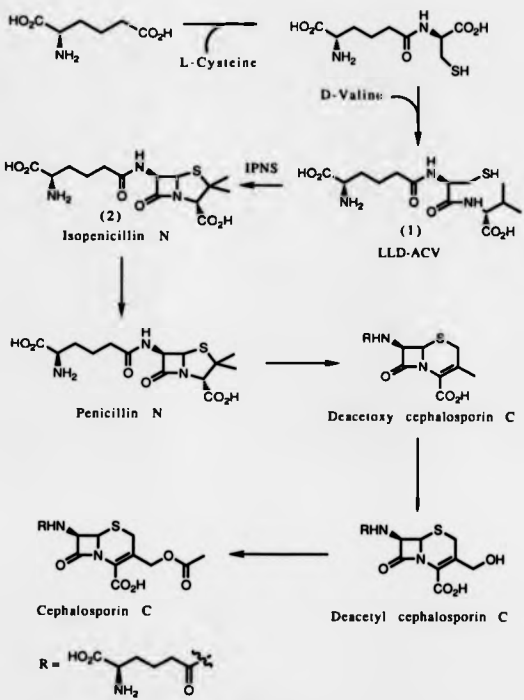
1.1 : Historical background.

Alexander Fleming first observed antibiosis between moulds in 1929. Considering the ingenuity of nature, the discovery that microorganisms wage chemical warfare to destroy competition should not be surprising. This activity has been seen in several different species of microbes. Man has since used these weapons in his own fight against pathological microorganisms. However the development of resistance to the original antibiotics has created the need for more active antibiotics.

The original and one of the most important of these groups are the penicillins. Penicillin N has been chemically synthesized but the chemical route cannot compete commercially with the efficiency of the producer microorganisms. There is thus a need to fully understand the biosynthetic pathway in order to exploit it.

1.2 : Biosynthetic pathway.

In *Cephalosporium acremonium* the biosynthetic pathway to penicillin N (and finally to cephalosporin C) has been elucidated (Scheme 1.1)[1,2].



Scheme 1.1

1.3 : Isopenicillin N synthase.

The crucial step in the biosynthesis is the cyclisation of the intact

(Arnstein) tripeptide (1) into isopenicillin N (2)[3,4]. Isopenicillin N differs from penicillin N by having the L- rather than the D-configuration in the amino adipoyl side chain. The enzyme responsible for this cyclisation (isopenicillin N synthase, IPNS) has been cloned[5] and highly purified[6,7] and found to be stable for at least two months at -20°C. IPNS is a non-heme iron oxidase enzyme with a molecular weight of 38kDa[8] and pI 5.05[9]. Though an X-ray crystal structure has not yet been obtained, the first 50 N-terminal amino acid sequence has been established[9].

IPNS has been found to exist in two states, an oxidized state (with a disulphide bridge) and an active reduced state (with free thiol groups), which can be interconverted and isolated[8]. These cysteinyl residues are not involved in the catalytic activity. When they were replaced with serine residues, by site-directed mutagenesis, the optical or EPR spectra for the similar IPNS-ACV-NO complex remained unchanged[10].

1.4 : Enzyme requirements.

There is a fundamental requirement for iron. The active site of IPNS contains one iron ion in the high (II) spin state. The use of different spectral techniques has demonstrated the capacity of IPNS to coordinate the ACV substrate and the co-substrate molecular oxygen to the iron simultaneously. The ACV appears to be bound to the iron by a single sulphydryl bond. This causes the iron to gain some Fe(III) character[10]. 10μM Fe(II) is sufficient for maximum activity. Removal of this Fe(II) by complexation with EDTA stopped cyclisation[6]. Enzymatic activity was also strongly inhibited by thiol blocking agents. Molecular oxygen consumption has a 1:1 stoichiometry with tripeptide

cyclisation[11], terminating as two molecules of water.

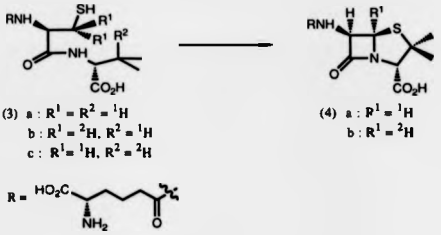
1.5 : Enzymatic synthesis of isopenicillin N.

Isopenicillin N (2) has a bicyclic structure with fused β-lactam and thiazolidine rings. The overall reaction is desaturative with four protons lost and two new bonds (C-N, C-S) formed, with retention of stereochemistry. Several intermediates originally proposed have been eliminated as being unstable in the reaction medium[12] or not being accepted as substrates or inhibitors (*ie* recovered unchanged)[13]. No evidence of any enzyme-free intermediates has been detected by nmr[14].

Incubating either unnatural and/or labelled substrates with IPNS has revealed a great deal about this complicated enzymic mechanism. These experiments have indicated the sequential cyclisation of the β-lactam and thiazolidine rings.

1.6 : Stereochemistry and requirements of β-lactam ring closure.

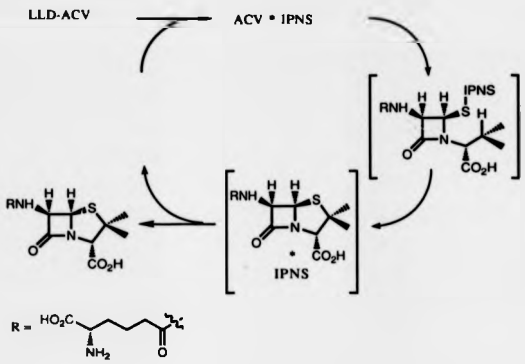
Direct evidence of the initial β-lactam ring closure has been gained with incubation of the specifically deuterated precursors (3a, b, c) (Scheme 1.2)[15,16].



Scheme 1.2

Incubation of a 1:1 mixture of (3a) and (3b) with IPNS resulted in the preferential formation of the unlabelled isopenicillin N (4a) in competition with (4b)[17]. This preference is a consequence of isotopic discrimination whereby the carbon-proton bond is more easily broken than the carbon-deuterium bond. No such discriminative cyclisation is seen in the incubation of a 1:1 mixture of precursors (3a) and (3c), both substrates are consumed at equal rates to give a single product (4a).

Isotopic discrimination in such a competitive mixed label incubation is a V_{max}/K_m effect and is observed only up to the first irreversible step[18]. This effect is present only with tripeptide 3b and not 3c. It therefore implies that the cleavage of the cysteinyl 3-H bond is the first such irreversible step. Accordingly this is indicative of the initial formation of an enzymatically bound monocyclic β-lactam intermediate (Scheme 1.3).

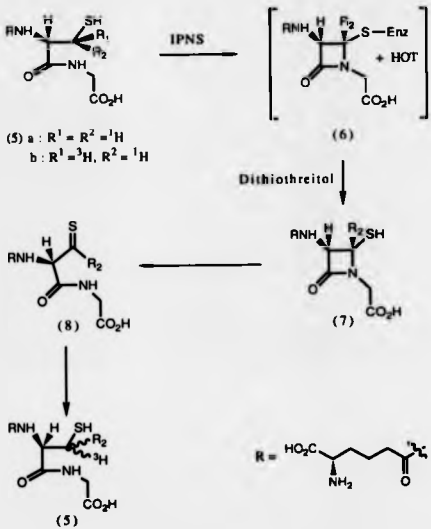


Scheme 1 . 3

Suicide substrates are compounds which mimic an enzyme's natural substrate and while being accepted as such result in the inactivation of the enzyme in the course of the transformation. The glycine analogue (5a) is such a suicide inhibitor[19]. Whilst it could form the proposed β-lactam ring intermediate, it is structurally incapable of forming the thiazolidine ring. The attempted cyclisation of (5a) by IPNS has thus been used to give information on the initial bond formation.

Incubation of the glycine containing tripeptide (5b) led to the release of tritium oxide in proportion to the inactivation of the enzyme (Scheme 1.4). The addition of dithiothreitol to the reaction medium resulted in the breakdown of the proposed enzyme substrate complex (6) to liberate the monocyclic β-lactam (7). The β-lactam ring then opened to give the thioaldehyde (8). Labelling can be introduced exclusively to the

C-3 cysteinyl position by reduction of thioaldehyde (8) by sodium borotritiide. Labelled tripeptide (5b) can thus be recovered from the incubation of unlabelled (5a).

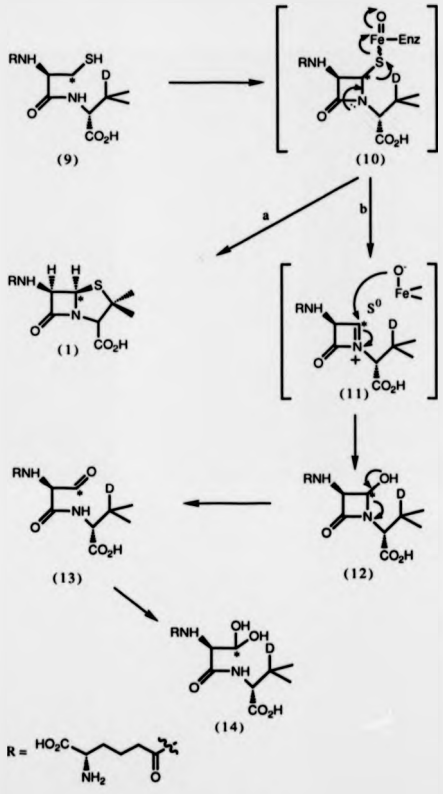


Scheme 1.4

Further evidence for the initial formation of the β-lactam ring was seen with the incubation of specifically labelled tripeptide (9) with IPNS[20]. Study of the enzymatic cyclisation with ^{13}C nmr revealed the presence of two peaks at approximately δ = 89 ppm. In addition to the

expected penam (1), a 'shunt metabolite' was also present. This shunt metabolite production was a result of the deuteration at the valinyl C-3 position. The primary isotope effect of this labelling decreased the efficiency at which the enzyme processed the presumed bound monocyclic intermediate (10). This resulted in the leakage of a metabolite identified as the geminal diol (aldehyde hydrate) (14) (Scheme 1.5).

The accumulation of this unexpected product was rationalised as follows. The cyclisation of the thiazolidine ring in the natural product required a carbon-hydrogen bond to be broken. In the deuterated precursor (9) this was slowed by the increased energy required to break the C-D bond of the valinyl residue. Consequently the alternative pathway b became significant. The intermediate (10) collapsed to the iminium ion (11) and atomic sulphur. Oxide attack on the iminium ion (11) gave the unstable hydroxy β-lactam (12). Hydrolysis of this entity yielded the aldehyde (13) which in the aqueous reaction medium was observed in the hydrated diol form (14). Indeed the efficiency of the IPNS was so decreased by the presence of this carbon-deuterium bond that the shunt metabolite (14) and isopenicillin N were formed in equal amounts.

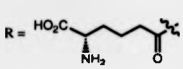

RNH
SH
D
NH
CO2H
(9)
O
Fe—Enz
S
N
(10)
a
b
H
S
(1)
O-
Fe
S0
(11)
OH
(12)
(13)
OH
(14)
R =
HO2C
NH2

Scheme 1 . 5

The cyclisation of the β-lactam ring is very stereospecific. Incubation of tripeptides stereospecifically labelled at the C-3 position of the cysteinyl residue (15a and 15b) with IPNS led to the complete loss of the 3-pro-S hydrogen and the complete retention of the 3-pro-R hydrogen of the cysteinyl residue in the penam (16)[21]. (Scheme 1.6). This was seen in tripeptide (15b) even though greater energy is required to break the carbon-tritium bond of the pro-S position relative to the carbon-proton bond of the pro-R position (Scheme 1.6).

(15) a : $H^R = {}^1H$, $H^S = {}^3H$
b : $H^R = {}^3H$, $H^S = {}^1H$

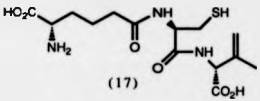
(16)



Scheme 1.6

1.7 : Stereochemistry and requirements of thiazolidine ring closure.

The closure of the thiazolidine ring by IPNS, at first, appeared to be equally stereospecific. Only the C-3 proton of the valinyl residue appeared to be abstracted[22]. The incubation of the didehydrovaline derivative (17) did not give isopenicillin N[23]. This suggested that formation of a dehydrovalinyl intermediate during cyclisation did not occur.

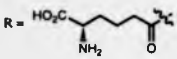


(17)

Incubation of natural substrates with specifically labelled valinyl residues (18a and 18b) cyclised with retention of the C-2 and C-3 configuration to give the appropriate penam (Scheme 1.7)[24].

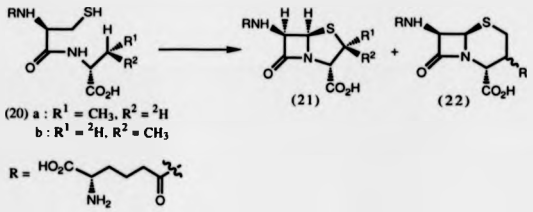
(18) a : $R^1 = C^2H_3$
b : $R^1 = {}^{13}CH_3$

(19)



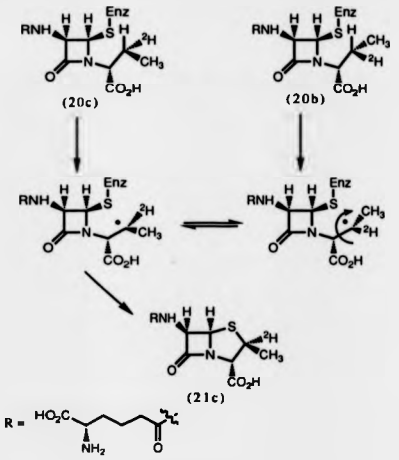
Scheme 1.7

A surprising result, however, was observed on incubation of the unnatural substrate containing an aminobutyrate residue (20a) with IPNS[25]. In addition to the expected penam product, a cepham (22) was also isolated. Furthermore incubation of the diastereomers (20a) and (20b), with a chiral C-3 centre, resulted in the formation of a single demethyl penam product with a β-methyl configuration (21b)[26]. There was no evidence of the presence of the α-epimer (21a) (Scheme 1.8).



Scheme 1.8

These anomalous results are in accordance with a free radical mechanism. Deprotonation at the C-3 position, with the breaking of the weaker C-H (over the stronger C-D bond) of both precursors (20b) and (20c) gave secondary radicals. These species were then sufficiently long lived to adopt the most favourable conformation with regard to the active site of the enzyme. In this case the β-conformer was formed (Scheme 1.9).



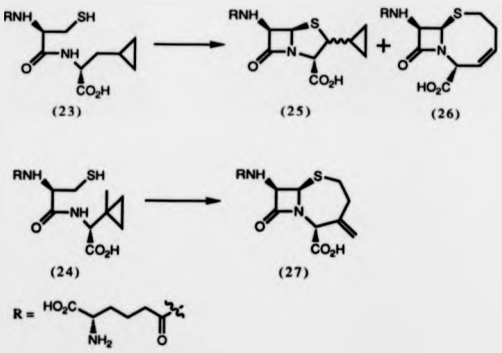
Scheme 1 . 9

This suggested mechanism was also in agreement with the formation of the cepham (22). There is no report of a cepham formed with the natural substrate. This was probably because the tertiary free radical that would be generated in the natural substrate is much more stable than the primary free radical that would form on the C-4 position. However in the tripeptide (20) the difference in energy between the C-3 secondary free radical and the C-4 primary free radical is apparently insufficient to favour its exclusive formation. Consequently, both cepham and penam products are formed. Cephams were also produced when isoleucine and norvaline containing tripeptides were incubated with IPNS[27].

A test for the free radical hypothesis would be to incubate a radical probe to trap the proposed intermediate if generated. Cyclopropyl carbinyl radicals are known to rearrange to but-3-enyl systems extremely fast, with a rate constant of 10^8 sec^{-1} (Scheme 1.10)[28].

Scheme 1 . 10

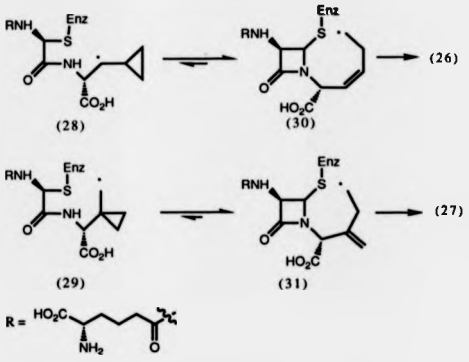
Tripeptides containing this cyclopropyl moiety (23) and (24) were incubated with IPNS (Scheme 1.11).



Scheme 1 . 11

Incubation of (23) resulted in the isolation of two products, a penam (25) and a cepham analogue (26), in the ratio of 1:3. Incubation of

(24) yielded a single product, a homocepham (27). The formation of both (26) and (27) is consistent with the free radical postulation. The free radicals of tripeptides (28) and (29) generated at the C-3 and C-4 positions respectively rearranged to give the open chain primary radicals (30) and (31). These then closed to the isolated cephams (26) and homocepham (27) with 6 and 7 membered rings respectively (Scheme 1.12).



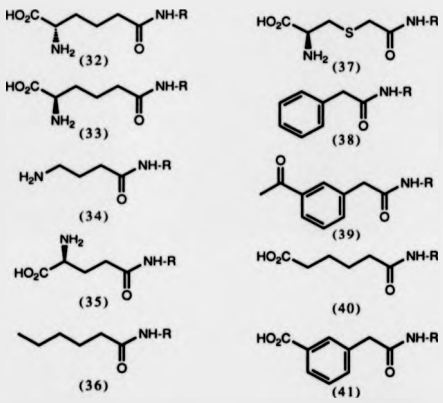
Scheme 1 . 12

1.8 : Substrate specificity of IPNS.

Systematic alteration of the specific residues has given an insight into the requirements and tolerances of IPNS. It has also indicated the range of possible enzymic products.

1.8.1 : Alteration of the aminoadipoyl residue.

The α-aminoadipoyl residue, while present, is not incorporated into the bicyclic structure. Its function, it appears, is to anchor the tripeptide to the enzyme and position it into the active site. A wide variety of tripeptides with differing residues in the primary position have been prepared and incubated with IPNS (Scheme 1.13)[29,30,31,32,33].



R = L-Cys-D-Val

Scheme 1 . 13

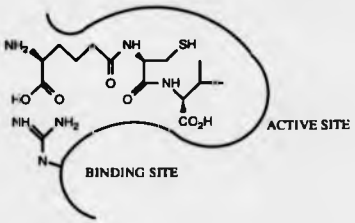
Tripeptides (34) (35) (36) and (39) were not transformed as they had low V_{max} values. The D-aminoadipoyl containing tripeptide (33) was

accepted for cyclisation, but at a lower rate. Analogues (37) and (40) were satisfactory substrates. These results signified that not only was the terminal L configuration not a requirement for cyclisation but that the terminal amino group did not even have to be present. It also indicated that to perform as an efficient substrate the 1^{o} residue must be of a length equivalent to a 6 carbon chain and terminate in a carboxylic acid group. The rigid transoid conformation of the *m*-carboxyphenylacetyl tripeptide (41) satisfied these requirements and in accordance with the above observations acted as an excellent substrate with a V_{max} comparable with that of the native tripeptide (Table 1.1).

Table 1.1 : K_m and V_{max} values of substrates 1, 34 and 37 for IPNS.

Substrate	Km (mM)	Vmax (μmol/min)
(1)	0.16	1.64
(33)	0.9	2.5×10^{-3}
(41)	0.8	0.8

By the use of isotopically labelled precursors it was established that none of the oxygen atoms of the aminoadipoyl residue were exchanged during the cyclisation[34]. This suggested that the carboxylic group is bound by electrostatic interaction to a basic residue in the IPNS peptide chain such as lysine or arginine. We can thus conceive the binding site to be a basic residue separated from the active site by a distance equivalent to the 6 carbon chain.

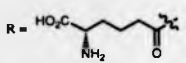


1.8.2 : Alteration of the cysteinyl residue.

As previously stated the β-lactam ring formation proceeded with the total loss of the cysteinyl 3-pro-S hydrogen atom and the total retention of the 3-pro-R hydrogen atom. The presence of this 3-pro-S hydrogen atom and the terminal thiol group was found to be an essential requirement for enzymatic activity. Provided these requirements were satisfied then the enzyme was tolerant to increases in the steric bulk of the 2^{o} residue[35].

(42) a : $R^1 = R^3 = H$, $R^2 = CH_3$
b : $R^1 = R^2 = H$, $R^3 = CH_3$
c : $R^2 = R^3 = H$, $R^1 = CH_3$
d : $R^2 = R^3 = H$, $R^1 = OCH_3$

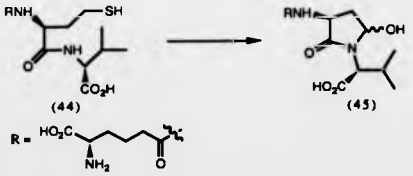
(43) a : $R^1 = H$, $R^3 = CH_3$
b : $R^1 = CH_3$, $R^2 = H$
c : $R^1 = OCH_3$, $R^2 = H$



Scheme 1.14

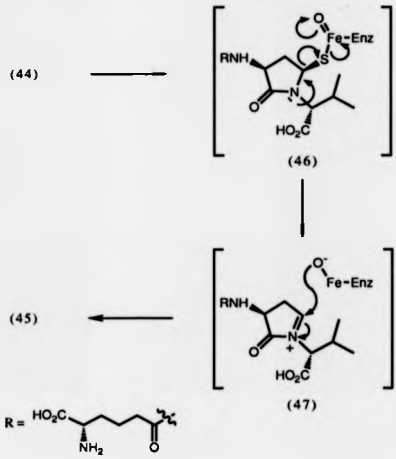
Incubation of tripeptide (42a), with a methyl group in the cysteinyl C-3 pro-R position of the natural substrate, resulted in the formation of the corresponding methyl penicillin (43a). However substituted tripeptide (42b), which lacked a C-3 (S) hydrogen, was not transformed[35]. Substrates (42c) and (42d) with α-methyl and α-methoxy cysteinyl residues respectively were both cyclised successfully into the corresponding penams (43b) and (43c) (Scheme 1.14)[36].

Incubation of homocysteinyl tripeptides (44) resulted in the exclusive formation of the monocyclic γ-lactam shunt metabolite (45) (Scheme 1.15)[37].



Scheme 1.15

The oxygen atom of the hydroxy group of (45) originated, in part at least, from the co-substrate, molecular oxygen. The proposed biosynthesis of the γ-lactam is as follows (Scheme 1.16).

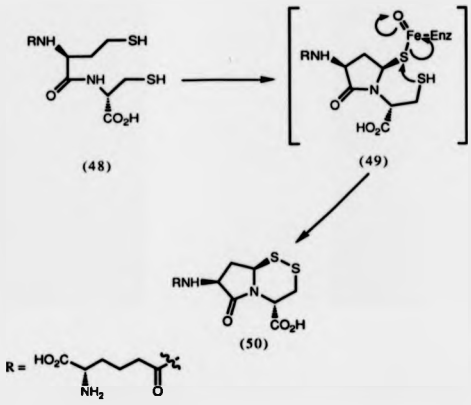


Scheme 1.16

Intermediate (47) was formed presumably because the substrate-enzyme complex (46) collapsed faster than the closure of the thiazolidine ring, with the release of atomic sulphur. This iminium ion was then attacked by the oxide of the iron-oxo centre to release the monocyclic γ-lactam (45).

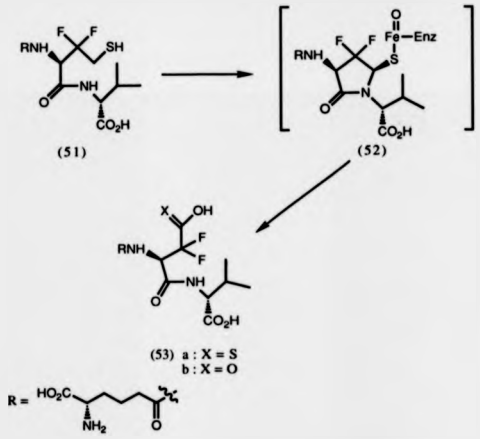
To prepare an bicyclic rather than a monocyclic γ-lactam a

nucleophile was included in the side chain of the tertiary residue. Substrates containing serine, allylglycine or aminobutyrate side chains yielded only the analogous monocyclic γ-lactam products. However the incubation of the tertiary cysteinyl residue (48) resulted in a single bicyclic disulphide γ-lactam product (50). There was no indication of any monocyclic γ-lactam or 5,5 bicyclic γ-lactam (Scheme 1.17)[38].



Scheme 1.17

The enzyme bound monocyclic intermediate (49) is proposed as having a sulphydryl bond between the homocysteinyl residue and the electron-withdrawing iron-oxo centre. Nucleophilic attack on the sulphur by the 3° cysteinyl thiol group breaks this Fe-S bond resulting in the exclusive generation of the bicyclic γ-lactam (50) (Scheme 1.17).



Scheme 1.18

Recently the difluoro analogue (51) was incubated with IPNS (Scheme 1.18)[39]. It was hoped that the electron-withdrawing fluorine atoms would inhibit the formation of positively charged iminium intermediate. The substrate-enzyme intermediate (52) would thus be sufficiently stabilised to enable a thiazolidine containing γ-lactam to form. However the only products isolated were the carboxylic acids (53a) and (53b). Product (53a) was seen to give (53b) at the pH of the incubation (the other oxygen coming from the co-substrate) indicating that (53a) is the only enzymatic carboxylate product. No cyclic products were detected. Thus the fluorine atoms do indeed appear to stabilise an enzyme-bound monocyclic intermediate (52), but instead of any cyclic

structure forming it is oxidised to the thiocarboxylic acid (53a) exclusively. This pathway is absent in the incubation of the unfluorinated analogue (48).

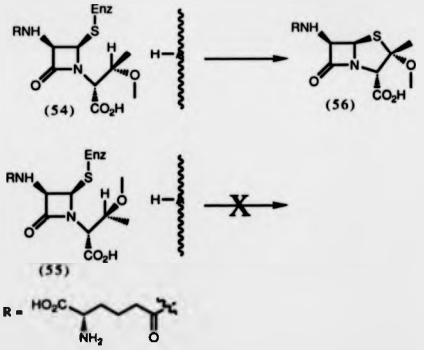
1.8.3 : Alteration of the valinyl residue.

A complicated relationship was observed between the alteration of the 3^{o} residue and the products recovered. In the natural substrate of the enzyme the thiazolidine ring closure proceeds with retention of configuration[24]. This appears to be totally a consequence of the active site topology which restricts rotation. Thus the isoleucine and alloisoleucine tripeptides also cyclise with retention[40]. When the side chains were sufficiently small to allow rotation, saturated groups tended to adopt the β configuration whilst the unsaturated groups adopted the α configuration. Thus both specifically deuterated aminobutyrate and the D-norvaline tripeptides had predominantly the β-configuration while the allenyl glycine and propargyl glycine tripeptides predominantly adopt the α-configuration. Allyl glycine tripeptide occupies an intermediate position. This observation possibly reflects the composition of the active site with saturated and unsaturated residues making up the β and α sites respectively. Cyanoglycine tripeptide gave a 1:1 ratio of α- and β-conformers indicating that the highly polar cyano group was unaffected by such influences (Table 1.2)[27,41].

Table 1.2 : 3° residue side chain v isomeric ratio of penams.

D-valine substitute	R group	α : β
D-norvaline	ethyl	>10:1
D-α-aminobutyrate	methyl	7:1
D-allylglycine	vinyl	4:1
D-propargylglycine	ethynyl	>1:15
D-cyanoglycine	cyano	1:1

IPNS catalysed the cyclisation of a tripeptide containing a D-(*O*-methyl)-*allo*threonine residue (54) to the expected penam (56); surprizingly however the D-(*O*-methyl)threonine containing tripeptide (55) was not accepted as a substrate for cyclisation (Scheme 1.19)[42,43].

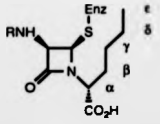


Scheme 1.19

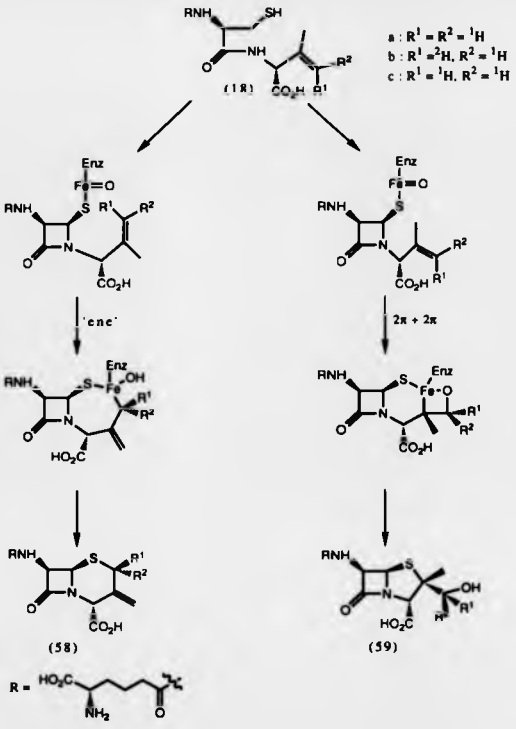
The stereochemistry of the C-3 position of the 3^{o} residue is the only difference between the substrates (54) and (55). The steric bulk of the methoxy group is comparable to the ethyl group of the successfully cyclised isoleucine analogue. Therefore this discrimination by the enzyme must be a result of substrate interference. Thus it must be the orientation adopted by the oxygen of the threonine precursor (55) within the active site that prohibits cyclisation. This does not occur with the similar *allo*threonine tripeptide (54). This observation reflects the sensitivity of the enzyme to the location of polar groups within the active pocket.

1.9 : Competing mechanisms of IPNS.

IPNS is curious in that it is an oxygenase enzyme that does not insert oxygen into its natural substrate. However incubation of substrates containing an unsaturated 3^{o} residue displayed two mechanisms in competition. Alongside the normal desaturative (-4H) mechanism, a competing monooxygenase activity is seen with a hydroxylative (-2H+1O) mechanism. The oxygen originated as the co-substrate molecular oxygen[44]. The balance between the two rival mechanisms depended on the position of the unsaturated bond in the tertiary residue of the substrate[45].



α β-Alkenes gave neither pathway as the products would presumably be too unstable. If the double bond was located between the βγ or the γδ bonds but not the δε bonds then the hydroxylative mechanism could occur in competition to the desaturative pathway. Thus the products of the enzymic cyclisation of the isodehydrovaline containing tripeptide (18) results from both mechanisms (Scheme 1.20)[46].

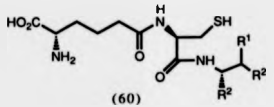


Scheme 1.20

The observed stereochemistry of products (58) and (59) is in accordance with the 'ene' reaction and a '*syn*' [2π+2π] cycloaddition

reaction respectively. This was then followed by reductive elimination with retention of configuration. This result suggested again that the proposed Fe-O centre is in close proximity to the second and third residues during cyclisation.

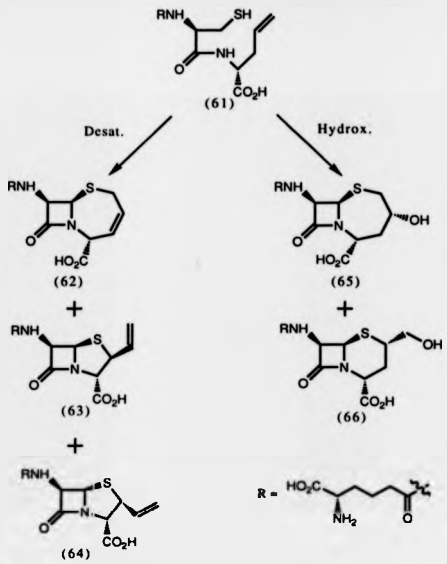
Tripeptides containing an allene side chain (60a) and (60b) resulted in products solely by a desaturative mechanism[47]. The absence of hydroxylative mechanism is possibly due to differences in electron density between the allene and the isolated double bond; the lower electron density of the allene is insufficient to enable electrophilic attack by the oxygen of the Fe-O centre.



(60)

a : R^1 = H, R^2 = CH=C=CH_2
b : R^1 = CH=C=CH_2, R^2 = H

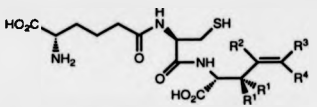
In contrast, the alkene derivative LLD-AC allylglycine (61) gave products by both routes. The enzymatic cyclisation results in the formation of different penam, homocepham, homoceph-3-em and hydroxyhomocepham products (Scheme 1.21)[48].



Ratio :	(62)	(63)	(64)	(65)	(66)
	10	4	1	5	2

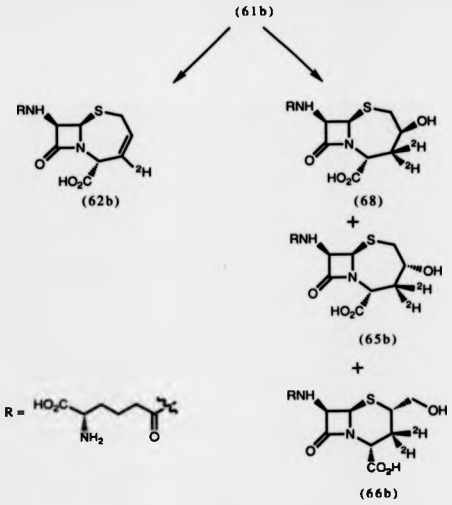
Scheme 1.21

Incubation of specifically labelled allylglycine tripeptides (61a-d) has indicated that more than two mechanisms were operating[49].



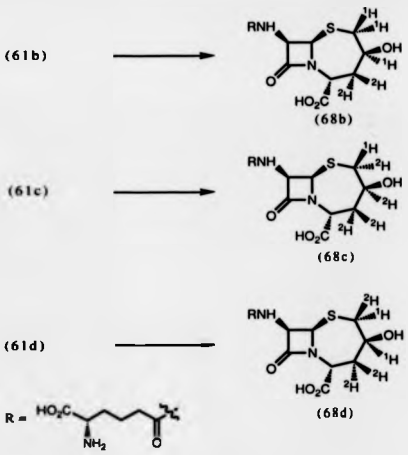
(61) :	R^1	R^2	R^3	R^4
a :	^1H	^1H	^1H	^1H
b :	^2H	^1H	^1H	^1H
c :	^2H	^2H	^2H	^1H
d :	^2H	^1H	^1H	^2H

Exploiting the known isotope effect, incubation of the analogue (61b), which was dideuterated at the C-3 position of the allyl glycine residue, with the enzyme gave products by the hydroxylative mechanism almost exclusively (Scheme 1.22).



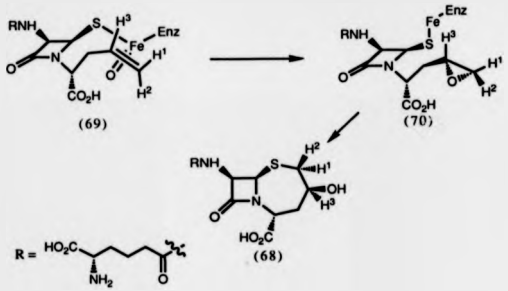
Scheme 1.22

Investigation of the formation of the hydroxyhomocepham (68) using derivatives (61c) and (61d) (specifically deuterated at the C-4 and C-5 positions) showed that inversion of configuration had occurred at the C-5 in relation to C-4.



Scheme 1.23

The inversion of the deuterium labelling of these products could be explained if epoxide formation or equivalent mechanism was operating[49] (Scheme 1.24). The cyclisation resulted in the opening of the epoxide ring of the enzyme bound intermediate (70) with inversion of the C-5 stereochemistry of the 3^{D} residue (Scheme 1.24). This observation is in contrast to the retention of configuration that is seen with the 'ene' reaction that results in the formation in (62).



Scheme 1.24

The competing mechanisms that are proposed to occur in the incubation of the allyl tripeptide can be identified as the following:

MECHANISM	PRODUCT.
1 : Hydrogen abstraction / recombination	(63)(64).
2 : Oxo-ene reaction	(62).
3 : [2π+2π] cycloaddition / reduction	(65)(66).
4 : Epoxide formation / inversion	(68).

Mechanisms 1 and 2 are desaturative whilst 3 and 4 are monooxygenative. The ratio between the competing mechanisms is probably a consequence of the geometry between the bound intermediate and the iron-oxene centre within the active site. The high energy of such a system giving rise to the complex pathways which then ensue.

1.10 : Summary.

1.10 : Summary.

The isopenicillin N synthase is a monooxidase enzyme that does not insert oxygen into its natural substrate. It is seen to exhibit very tight stereochemical control in the β-lactam ring formation while being tolerant of changes in steric bulk. The thiazolidine ring closure is seen to be much more loosely controlled with a variety of mechanisms expressed depending on the substrate.

CHAPTER 2

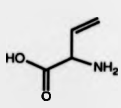
VINYLGLYCINE, ITS METHODS OF PREPARATION AND THE NEBER REARRANGEMENT.

2.1 : Biological uses of vinylglycine.

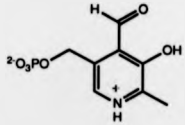
Vinylglycine (71), the simplest of the β,γ-unsaturated α-amino acid series, has been isolated from mushrooms[50]. As with other non-proteinogenic amino acids it is the possible biological applications that provoked interest in vinyl glycine. As a consequence it has been shown to be a specific inhibitor of photorespiration[51] and a plant growth regulator[52]. It has also displays anti-bacterial properties against *Bacillus subtilis* and *Escherichia coli*[53].

2.2 : Mechanism of inhibition of pyridoxal-phosphate dependent enzymes.

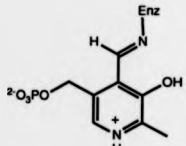
However it is the inhibition of a wide range of pyridoxal-5-phosphate (PLP) (72) dependent enzymes that provokes greatest interest. These enzymes include aspartate aminotransferase[54], alanine racemase[55], serine:glyoxylate aminotransferase[56], heart α-ketoglutarate dehydrogenase[57] and other PLP dependent enzymes[58]. PLP is an enzyme co-factor which in the absence of the substrate forms a Schiff-base with a ε-NH_2 of a specific lysine residue within the active site of the enzyme (73). The α-amino substituent of the substrate displaces the lysine of the enzyme prior to the biotransformation. The covalent Schiff base PLP-substrate intermediate (74) is bound by non-covalent forces of attraction within the active site (Scheme 2.1). After the biotransformation the Schiff base is hydrolysed releasing the product. PLP also stabilises negatively charged intermediates[59].



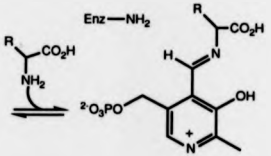
(71)



(72)



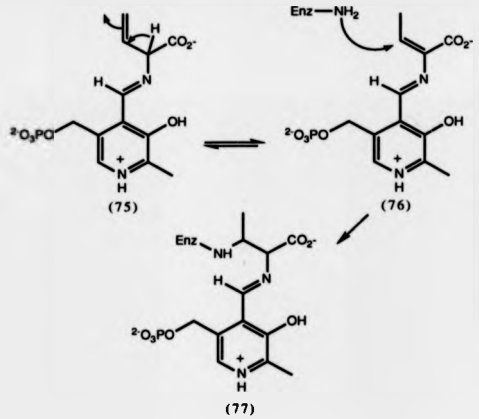
(73)



(74)

Scheme 2.1

Rando has given a possible mechanism for the inhibition of aspartate aminotransferase[60]. The enzyme deprotonates the α-hydrogen of the PLP bound vinylglycine intermediate (75) resulting in isomerisation of the double bond to give the conjugated intermediate (76). The active site lysine then reacts with a Michael addition to give an alkylated lysine residue which destroys the catalytic activity of the enzyme (77) (Scheme 2.2).



Scheme 2.2

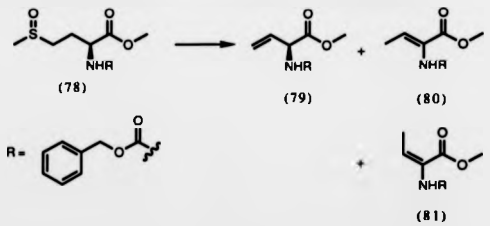
2.3 : Synthetic preparations of vinylglycine.

Synthetic vinylglycine was first prepared by Larsen *et al* in 1974[61]. In this report vinylglycine was prepared in extremely low (1%) yield by two methods; the amination and saponification of ethyl-2-bromo-3-butenoate and by a Strecker reaction from acrolein. Over the last 10 years improved methods for the preparation of both enantiomerically pure and racemic vinylglycine have been published. A brief synopsis of these procedures is presented in the following pages. The method of Sawada *et al*[62] for the preparation of specifically deuterium labelled racemic vinylglycine is discussed in the Chapter 3 and will not be examined here.

In 1984 Greenlee also used the Strecker reaction for his preparation of racemic vinylglycine using trimethylsilyl cyanide instead of potassium cyanide. A slightly better yield of 7% was achieved from acrolein. The application of this method to prepare other analogues of vinylglycine was more successful with yields ranging from 15 to 68%[63].

2.3.1 : Oxidative and thermal degradation of amino acids.

Oxidative degradation of amino acid derivatives has been employed in several preparations of vinylglycine. It has the advantage in that the stereochemistry of the original amino acid can be preserved in the key step. Afzali-Ardakani and Rapaport used this approach in their synthesis of L-vinylglycine from L-methionine with 54% yield in five steps (Scheme 2.3)[64]. The thermolysis of the methionine sulphoxide derivative (78) gave the protected vinylglycine (79).



Scheme 2.3

The temperature and pressure of the thermolysis used in the sulphoxide had a significant effect on the yield of product and the degree of isomerisation to the α,β-unsaturated isomer (80 and 81). The

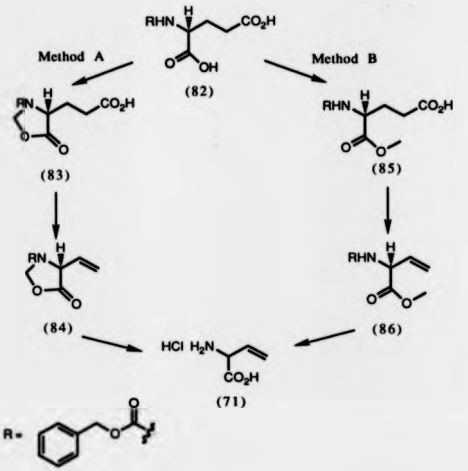
optimum conditions gave 90% yield with 5% isomerisation to give the *cis* conjugated by-product (80). This result gives an indication of one of the problems associated with the synthesis of the vinylglycine; namely that of the isomerism of the double bond due to the labile α-proton.

In 1989 Meffre *et al* also used thermolysis of the sulphoxide (78) for the preparation of the corresponding vinyl glycine derivative[65]. In this method 1,2 dichloro benzene was used to dissolve the sulphoxide at 160°C for 3.5 hours. Again temperature was found to be critical to limit the degree of isomerisation. The yield also depended on the amount of the sulphenic trapping agent calcium carbonate used. However the elimination reaction is relatively inefficient with nearly half the starting sulphoxide recovered from the reaction mixture, giving an overall yield of only 20% of the vinylglycine derivative in three steps.

Belokon *et al* report the production of optically enhanced vinylglycine (58% ee) in 45% yield from racemic methionine[66]. The chiral complex of (*S*)-*o*-[*N*-(*N*-benzylprolyl)amino] benzophenone and nickel (II) enantioselected only the L-isomers to form an optically pure Schiffs base. Oxidation and thermal elimination at 180°C of the diastereomerically pure nickel (II) methionine complex gave a mixture containing the nickel (II) vinylglycine complex which acid treatment gave the vinylglycine derivative. The mechanism is assumed to be similar to the Rapoport mechanism. The poor optical purity was attributed to the reaction workup rather than the racemisation of the complex.

Hanessian and Sahoo used the decarboxylative elimination of L-glutamate derivatives, facilitated by lead tetraacetate and cupric ion, to

prepare optically pure vinylglycine (Scheme 2.4)[67]. Two routes were devised with overall yields of 45% from L-benzyloxycarbonylglutamic acid.



Scheme 2.4

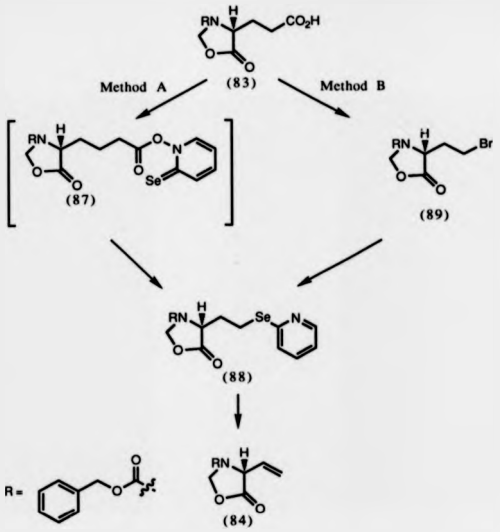
In method A the α-carboxylic acid is neatly protected as the readily prepared formaldehyde adduct (83) and treated with the lead tetraacetate to effect the decarboxylation to the vinylglycine (84). The yield for this step is stated as approximately 50% however when repeating the step in this laboratory an almost quantitative yield was achieved. Thus this convenient and practical method can have a much

higher overall yield than that reported. Method B merely involves treating the formaldehyde adduct (84) with methoxide to produce the α-methyl ester of *N*-benzyloxycarbonyl-glutamate (85) prior to the decarboxylation step. Deprotection by acid hydrolysis gave the vinylglycine hydrochloride (71).

2.3.2 : Use of selenium chemistry in the synthesis of vinylglycine.

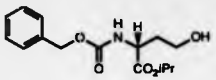
The relatively high temperatures required by the above methods are inconvenient for large scale synthesis. Selenoxides readily undergo a *syn* elimination at or around room temperature. Barton *et al* developed two routes involving selenoxides in the formation of L-vinylglycine from protected glutamic acid[68].

Method A in the preparation of vinylglycine consisted of three steps with 45% overall yield. The glutamic selenohydroxamic ester (87) was prepared from selenohydroxamic acid and rapidly rearranged to give the alkylselenide (88) with the loss of carbon dioxide. Ozonolysis of this compound and *in situ* elimination gave the vinylglycine (83). Method B involved four steps with 52% overall yield. In this instance the use of selenohydroxamic acid was avoided by the preparation of the phenyl selenide (88) *via* the bromide (89). (Scheme 2.5).



Scheme 2.5

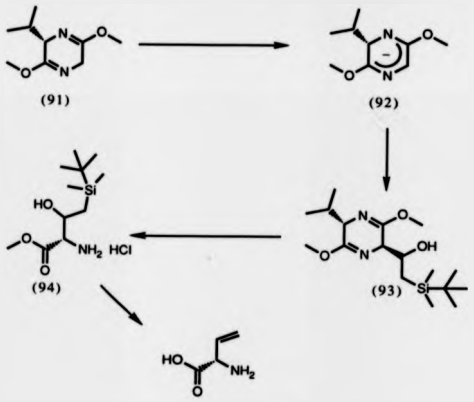
Similarly Pellicciari *et al* prepared optically pure vinylglycine from the *in situ* oxidation and elimination of the 2-nitrophenyl-selenocyanate adduct of protected homoserine (90) in three steps with 49% yield[69].



(90)

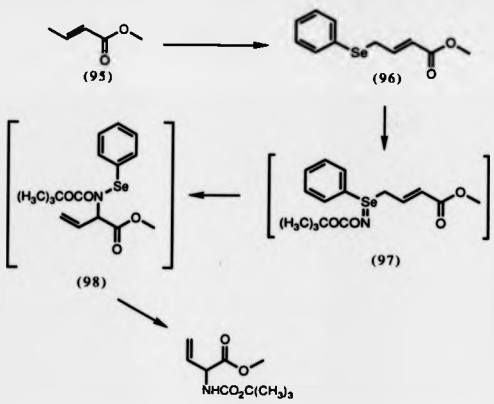
2.3.3 : synthesis via heterocyclic compounds.

Schollkopf *et al* used heterocyclic chemistry to prepare enantiomerically pure vinylglycine (Scheme 2.6)[70]. Heterocycle (91) was prepared from a chiral auxiliary (methyl L-valinate) and glycine methyl ester. Deprotonation of the acidic C-H by butyl lithium formed a rigid and planar anion (92). The asymmetric electrophilic attack by 2-[dimethyl t-butyl silyl] ethanal (a masked ethenyl group) on the planar anion results in a single diastereomer (93). Acid hydrolysis of this product cleaved the imino ether linkages to give the isolatable free hydroxy silyl (94). Acid and ion exchange treatment gave vinylglycine (71) in 25% overall yield. This method can also be used for the preparation of other β,γ-unsaturated amino acids[70,71].



Scheme 2.6

Shea *et al* also used selenium chemistry in their preparation of racemic vinylglycine in a four step synthesis (Scheme 2.7)[72]. The γ-bromination and phenylselenide displacement of methyl 2-butenoate (95) gave the selenide (96). The key stage of the synthesis was the *N*-chlorosucinimide treatment of this intermediate to the selenide (97) which underwent a [2,3] sigmatropic rearrangement to the vinylglycine (98). Acid treatment gave the protected vinyl glycine (99) with overall yield of 18% .



Scheme 2.7

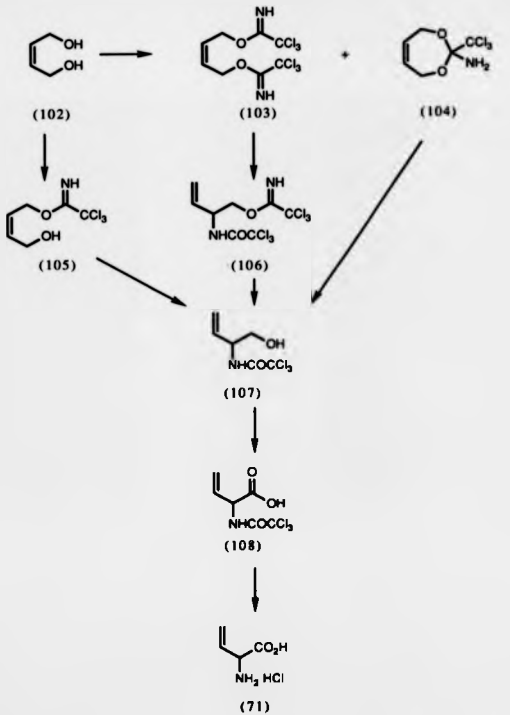
2.3.4 : Use of glycine equivalents.

The use of electrophilic glycine equivalents for the synthesis of vinyl glycine has been investigated by several groups[73,74,75]. All involved the initial preparation of chloro or bromo glycine derivatives (100) followed by reaction with a vinyl Grignard reagent. The derivative (100) can be considered as the carbocation equivalent (101). This method gives good yields of a wide range of racemic β,γ-unsaturated amino acids.

(100) (101)

2.3.5 : Sigmatropic rearrangements to vinylglycine.

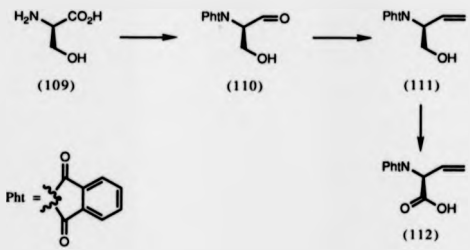
Vyas *et al* made use of the sigmatropic rearrangement in their vinyl glycine preparation from the inexpensive (Z)-2-butene-1,4-diol (102) (Scheme 2.8)[76]. Imidates (103), (104) and (105) were prepared under a variety of conditions. The thermal [3,3] sigmatropic rearrangement of these intermediates at 180°C gave the alcohol (107). The Jones oxidation and acid hydrolysis of the compound (107) gave vinyl glycine hydrochloride (71). The poor yield from the hydrolysis step, only 44%, caused the overall yield from butenediol to be only 26%. This could presumably be increased when the efficiency of hydrolysis is improved.


OH
OH
NH
O
CCl3
O
CCl3
NH
O
O
CCl3
NH2
(102)
(103)
(104)
NH
O
CCl3
OH
NH
O
CCl3
NHCOCCl3
(105)
(106)
OH
NHCOCCl3
(107)
O
OH
NHCOCCl3
(108)
CO2H
NH2 HCl
(71)

Scheme 2.8

2.3.6 : Use of Wittig reagents.

The most recently published synthesis involves the use of a Wittig reaction with the aldehyde (110) followed by oxidation (Scheme 2.9)[77]. In this manner optically pure vinylglycine can be prepared in a five step synthesis starting from serine of the opposite configuration. The overall synthesis however proceeds with a low overall yield.

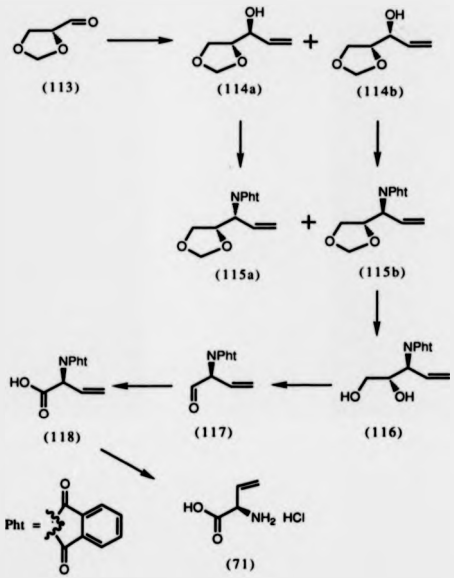


Scheme 2.9

2.3.7 : Use of D-mannitol.

Mulzer *et al* used D-mannitol as an chiral educt in the synthesis of D-vinylglycine (97% ee) in six steps (Scheme 2.10)[78]. Erythro-selective addition of organometal reagents to the starting materials gave the vinyl alcohols (114a and b). The diastereomeric phthalates (115a and b), prepared by Mitsunobu inversion, were separable by crystallisation. Deprotection to the diol (116) followed by a Jones oxidation and further

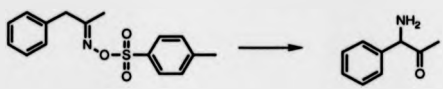
deprotection, by refluxing with hydrazine, gave vinylglycine in 10% overall yield.



Scheme 2.10

2.4 : Historical Background of the Neber rearrangement.

The 'Neber rearrangement' is the general name given to the reaction observed when oxime arylsulphonates are treated with a base. The nitrogen is seen to undergo a 1,2 migration, with the loss of the tosylate ion, to yield an α-amino ketal. It can thus be an effective method for the preparation of α-amino ketones. The reaction was first observed in 1929 by F.W. Neber and A. von Freidolheim when investigating the Beckmann rearrangement[79]. Their alkoxide treatment of phenyl propanone oxime tosylate (119) gave 1-phenyl-1-amino-propanone (120). This original example is demonstrative of a typical migration.



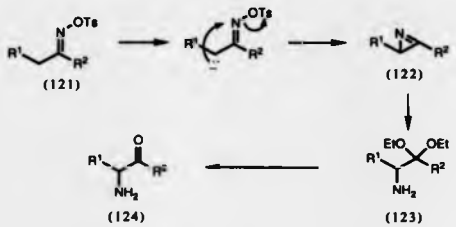
(119) (120)

Over the following years they extended this reaction to the preparation of both straight chain[80] and cyclic amino ketones[81]. The mechanism originally proposed by Neber is generally that quoted today[82] (Scheme 2.11).

2.5 : Mechanism of the Neber rearrangement .

In the Neber mechanism[82] the alkoxide removed the acidic proton from the α-methylene of the oxime (121) (Scheme 2.11). The anion then closed to form an azacyclopropene (azirine) ring intermediate (122) with concomitant loss of the tosylate group. Solvolysis of the azirine ring resulted in the ketal (123). The amino ketone (124) is recovered after acid

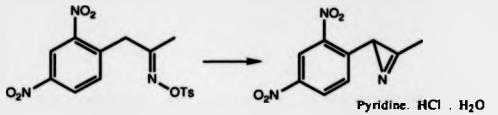
treatment. The lack of isomeric amino ketones indicated that tautomerisation was absent in the azirine ring of intermediate (122).



R^1 = Aryl, R^2 = aryl, alkyl, etc.

Scheme 2.11

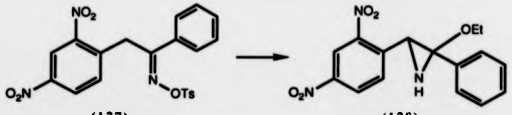
The presence of the azirine ring is remarkable. This three membered unsaturated ring is a highly strained and therefore unstable entity. Neber cited the base treatment of 1-(2,4-dinitrophenyl) propanone oxime tosylate (125) as evidence of its participation in the mechanism. There were two consequences of the presence of these electron withdrawing nitro groups which make this oxime exceptional. Firstly the acidity of the methylene protons was increased such that pyridine was sufficiently basic to initiate the reaction. Secondly, and more importantly, the stability of the azirine intermediate was enhanced due to the increased resonance structures provided by the nitro groups. Thus the otherwise fleeting azirine intermediate was isolated as the hydrochloride of a pyridine-water complex (126)[80].



(125) (126)

2.5.1 : Hatch and Cram proposals.

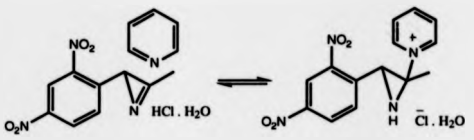
Other mechanisms have been proposed to explain the migration. Hatch and Cram confirmed the Neber findings twenty five years after the original reports[83]. Lithium aluminium hydride reduction of the intermediate (126) gave the corresponding aziridine. However they suggested that the stability of the azirine moiety of intermediate (126) was an extreme case and might not be representative. They studied examples thought to be more typical of the normal oximes. They isolated intermediate (128) from the alkoxide treatment of desoxybenzoin oxime tosylate (127). From this they reasoned that the strained azirine need not be present. Instead it was suggested that the reaction was a base catalysed 1,3 elimination superimposed on a 1,2 addition reaction. This pathway would give their isolated alkoxy ethylenimine (128)[84].



(127) (128)

To make their findings compatible with the isolation of intermediate (126) they proposed that the pyridine-water complex was a

resonance form of the structure (129) similar to the structure that they had suggested.



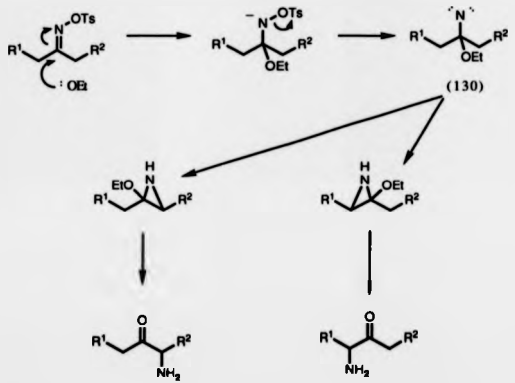
(126) (129)

Products from stereospecific Beckmann rearrangement side reactions indicated that the equilibration of the oxime bond did not occur under the reaction conditions. Surprisingly in the Neber rearrangement the configuration of the oxime bond did not appear to influence the direction of nitrogen migration. Thus both *cis* and *trans* oximes gave the same amino ketone[85]. When two differing α-methylenes were available the reaction proceeded in a *trans* direction[84]. The single exception is 1-(2-nitrophenyl)propanone-β-oxime tosylate. This anomaly possibly results from some interaction between the nitrogen of the oxime and the *ortho*-nitro group that prevent the normal steric course.

This observation is very unexpected. A strong and unfavourable steric interference would be expected between the attacking anion and the *cis* tosylate group which should markedly influence the reaction. This suggests two further mechanistic possibilities; *ie* saturated and unsaturated nitrene intermediates.

2.5.2 : Presence of nitrenes?

In addition to the above observation, nitrenes would explain the lack of stereospecificity of the Neber rearrangement in contrast to that seen in the Beckmann rearrangement. These could also account for the fact that rearrangements involving substituted nitrogens have not been reported. A saturated nitrene intermediate (130) could arise from a direct base attack on the carbon-nitrogen double bond. Then, after the elimination of the negatively charged tosylate group, a singly bonded neutral nitrogen would result (Scheme 2.12).

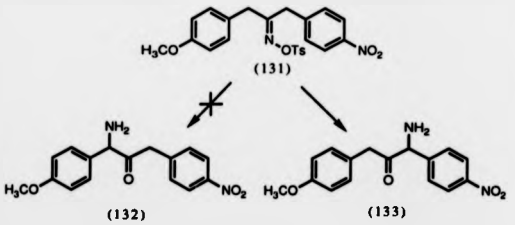


R^1 = aryl, alkyl, etc.
R^2 = aryl, alkyl, etc.

Scheme 2.12

This species (130), in common with other nitrenes and carbenes, should display little selectivity in the insertion reaction with the adjacent carbon-hydrogen bonds. Where two discernible carbon-proton bonds are present the electrophilic nature of the nitrene should favour attack at the centre with higher electron density.

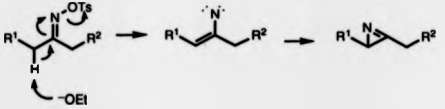
1-(4-methoxyphenyl)-3-(4-nitrophenyl)propanone oxime tosylate (131) possesses such distinguishable methylenes. The C-1 methylene with the attached methoxy substituted phenyl ring is electron rich. In contrast the nitro phenyl substituted C-3 methylene is relatively electron deficient with acidic protons. Only the acidic protons of the C-3 methylene are seen to be removed. Alkoxide treatment of oxime tosylate (131) results in the rearrangement to a single amino ketone (133) with a complete absence of the isomeric product (132)[85]. This finding eliminates the possibility of a saturated nitrene being present as an intermediate during the nitrogen migration (Scheme 2.13).



Scheme 2.13

The presence of an unsaturated nitrene is however a possibility[86]. The base deprotonation and the loss of the tosylate could lead to the

formation of the unsaturated nitrene intermediate which could then close to the azirine ring (Scheme 2.14). There is an example of such intermediates giving rise to an azirine ring[87]. The presence of an unsaturated nitrene could also explain why the configuration of the oxime has no influence on the product formed and the non-stereospecific nature of the rearrangement.



R^1 = aryl, alkyl, etc.
R^2 = aryl, alkyl, etc.

Scheme 2.14

Although the mechanism has not been conclusively established yet the Neber rearrangements have been used over the years where conventional methods have failed. In addition to the tosyl group other leaving groups have also been employed successfully. These include the use of chloride etc[88,89,90].

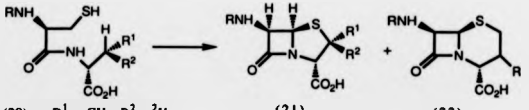
CHAPTER THREE.

INVESTIGATIONS INTO THE STEREOCHEMISTRY OF THE ISOPENICILLIN N SYNTHASE CATALYSED CYCLISATION OF AN UNNATURAL SPECIFICALLY LABELLED PRECURSOR.

3.1 : Background.

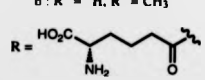
The mechanism by which the enzymatic thiazolidine ring closure occurs during penicillin biosynthesis is still not completely understood. As stated in the introduction the proposed mechanism involves a rotation restricted free radical intermediate. As a consequence the natural substrate (1) undergoes cyclisation with retention of stereochemistry to the penam (2).

In the cyclisation of the aminobutyrate tripeptides (20a and 20b) the smaller size of the radical intermediate means that it is able to rotate before cyclisation occurs. This results in the isolation of a single β-demethyl isopenicillin N (21b) product from both precursors (Scheme 3.1)[25].



(20) a : $R^1 = CH_3$, $R^2 = {}^2H$
b : $R^1 = {}^2H$, $R^2 = CH_3$

(21) (22)

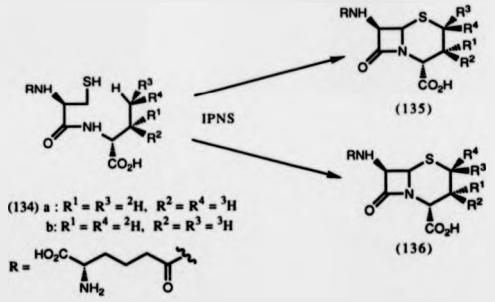


Scheme 3.1

However when the proton is abstracted from the C-4 position of the unnatural substrates (21a and 21b) an additional product, a cepham (22) was also recovered.

3.2 : Project objective.

The objective of this project was to examine the stereochemistry of the enzymatic formation of cephams. This involved the preparation of the stereospecifically labelled tripeptides (134a) and (134b) and incubating them with IPNS. Cepham (135 and 136) formation would be favoured over penam formation, as breaking the relatively weak carbon-proton bond of the C-4 position would be preferred to breaking the carbon-deuterium and carbon-tritium bonds (Scheme 3.2).



Scheme 3.2

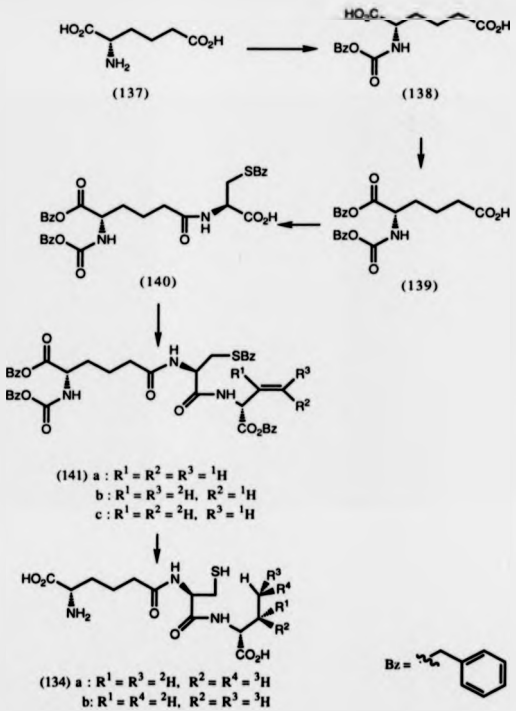
Tritium nmr could have been used to examine the stereochemistry of the cyclisation. The spectra of the cepham product(s) would have been

simple and would have shown only two signals corresponding to the two tritium atoms in the product. Examination of the chemical shifts and the coupling constants in the spectra could then have been used to see if the cyclisation had proceeded with retention or inversion (yielding a single cepham 135 *or* 136), or loss of stereochemistry (yielding a mixture of 135 *and* 136).

In one case (retention), the substrate 134a would give a product with a *cis*-arrangement of tritium atoms whereas in the other case (inversion), a *trans*-arrangement would result. The complementary result would be obtained with the substrate 134b. These possibilities would be distinguishable on the basis of the different coupling constants for the *cis* and *trans* isotopomers.

3.3 : General strategy.

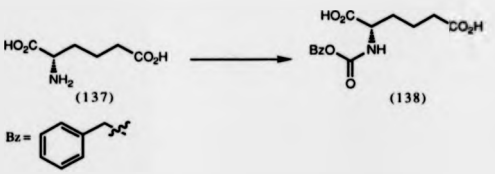
The proposed pathway for the preparation of the specifically labelled substrates was a five step synthesis involving specifically deuterated vinylglycine and benzyl protecting groups (Scheme 3.3). All benzyl protecting groups could have been removed in a single step with sodium in liquid ammonia[91]. Tritiation of the specifically deuterated vinylglycine derivatives in the penultimate step of the pathway would have given the product in high radiopurity at a late stage of the synthesis.


(137)
(138)
(139)
(140)
(141) a : R1 = R2 = R3 = 1H
b : R1 = R3 = 2H, R2 = 1H
c : R1 = R2 = 2H, R3 = 1H
(134) a : R1 = R3 = 2H, R2 = R4 = 3H
b: R1 = R4 = 2H, R2 = R3 = 3H
Bz =

Scheme 3.3

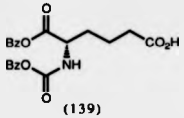
3.4 : Synthesis of benzyl *N*-benzyloxycarbonyl-L-α-aminoadipate (139).

The preparation of the *N*-benzyloxycarbonyl-L-α-aminoadipic acid (138) was a straight forward reaction with benzyl chloroformate and L-α-aminoadipic acid in a biphasic mixture of toluene and water at 0°C and was accomplished in high yield (85%) (Scheme 3.4)[92].



Conditions : Benzyl chloroformate, toluene, acetone, water, sodium hydroxide, 0°C, 2hr.

Scheme 3.4



(139)

The preparation of α-benzyl *N*-benzyloxycarbonyl-L-α-aminoadipate (139) is more complicated due to the presence of two carboxylic acid groups. The α-benzyl ester (139) is a key intermediate in the synthesis of a wide range of unnatural substrates for IPNS. There have been many efforts to synthesize this from α-aminoadipic acid and other amino acids[92,93,94]. The usual method of preparing it from the parent amino acid is by the Williamson synthesis. This involves preparing the mono caesium salt of the α-carboxylic acid group of

N-benzyloxycarbonyl-L-α-aminoadipic acid and reacting with benzyl bromide to give the desired product. When we attempted to prepare the product by this method it was found to be unreliable and readily formed dibenzyl ester instead. Attempts to specifically remove the ω-carboxylic acid benzyl group by a range of enzymes were not successful.

This method was clearly not a reliable one for the repeated preparation of this intermediate in high yields. Ideally we require a method that could only give the α-benzyl mono ester as the sole product.

Enzymes are well known for being both stereo and regioselective in their reactions. Thus, in principle, an enzymatic method would be ideal to protect specifically the α-carboxylic acid group of the amino acid α-aminoadipate. Papain is an inexpensive thiol protease derived from the papaya plant. It has an active site thiol residue and the catalysis proceeds through a thioester intermediate[95]. It has a wide substrate range and is stereospecific for the L-isomer of amino acid substrates. It has previously been used by Cantacuzene *et al* to resolve amino acids into the L-ester and the D-free acid[96]. Papain was therefore an ideal candidate for the preparation of the mono ester (139).

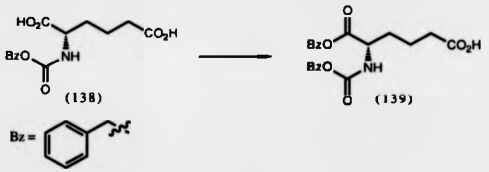
The esterification of the substrate by papain, like all enzymatic esterifications, is a reversible equilibrium reaction; which increases both the forward and reverse rates of hydrolysis equally. In an aqueous medium the presence of approximately 55 molar water results in an unfavourable equilibrium with respect to ester formation. However, biphasic systems have been used to overcome this limitation[96]. If the ester product of the reaction is preferentially partitioned into the organic phase relative to the substrate then the equilibrium is shifted

towards ester formation. The equilibrium constant for such a reaction, $K_{biphasic}$, in a two phase system, is related to the equilibrium constant in water, the partition coefficients and the volume of the phases as follows[97].

$$K_{biphasic} = [B]/[A] = K_w \left[\frac{1 + P_B \frac{V_{org}}{V_w}}{1 + P_A \frac{V_{org}}{V_w}} \right]$$

[A] and [B] represent the concentrations of the reactant and the product respectively, K_w is the equilibrium constant of the reaction in the aqueous phase, P_A and P_B are the partition coefficients of the reactant and the product respectively between the two phases and V the volume of the phase.

For the papain-catalysed α-esterification of *N*-benzyloxycarbonyl-L-α-aminoadipic acid (138), a biphasic mixture of dichloromethane and 0.1 M citrate / phosphate buffer (pH 4.2) was used. Cysteine and ethylene diaminotetraacetic acid tetra sodium salt were added to the reaction medium in order to protect the thiol residue within the active site of the papain and to complex out any heavy metal ions present in the solution (Scheme 3.5).



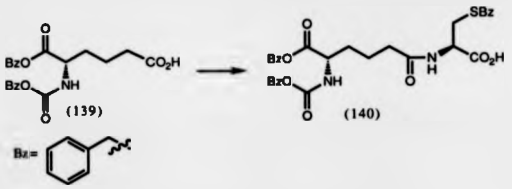
Conditions : papain, dichloromethane, benzyl alcohol, citrate / phosphate buffer, pH 4.2, 37°C, 2 days.

Scheme 3.5

The mixture was stirred at 37°C for two days until the starting material had disappeared (tlc). The product was extracted with ethyl acetate and the combined organic layers were worked up to give an oily residue of product and excess benzyl alcohol, which were separated by flash chromatography. Initial elution with a petroleum ether / ethyl acetate 20 : 1 mixture removed the benzyl alcohol. The product was then obtained by elution with petroleum ether / ethyl acetate 3 : 1 mixture in 66% yield. The product was recrystallised from ethyl acetate / petroleum ether to give the pure monobenzyl ester (139).

3.5 : Preparation of benzyl-protected aminoadipyl cysteine (140).

The dipeptide (140) was prepared by the addition of isobutyl chloroformate to a stirred solution of the protected aminoadipate to form the mixed anhydride[98]. Addition of the S-benzyl cysteine resulted in the formation of the dipeptide which was isolated by extraction in 54% yield and used without further purification (Scheme 3.6). The light sensitive nature of the dipeptide, which resulted in the loss of the *S*-benzyl group, necessitated its storage in a cold and dark environment.



Conditions : *iso*butyl chloroformate, THF,
S-benzyl-cysteine, triethylamine, 0°C

Scheme 3.6

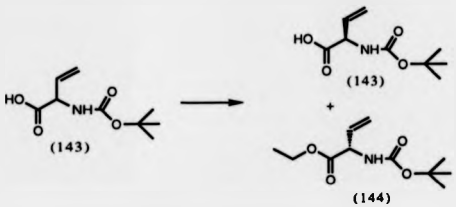
3.6 : Preparation of benzyl D-vinylglycinate *p*-toluenesulphonic acid salt (142).

The delicate nature of the vinylglycine precluded application of the normal method for preparing benzyl esters of amino acids by refluxing in benzene with benzyl alcohol and *p*-toluene sulphonic acid. Thus a gentle method of preparing the benzyl ester *p*-toluenesulphonic salt of vinylglycine (142) was required.

The *N*-*t*-butyloxycarbonyl (Boc) vinylglycine (143) derivative was prepared by the addition of 1.2 equivalents of di-*t*-butyl-dicarbonate to a solution of vinylglycine in 1,4-dioxane / 5% aqueous sodium bicarbonate / water 2 : 1 : 1 in high yield (95%)[99].

As the D-isomer only is required in the tripeptide (134), the enzymatic removal of the L-isomer at this stage was planned, as this would avoid wasting half the valuable dipeptide and ease the chromatographic separation of the LLL-tripeptide from the LLD-

tripeptide. Esterification was achieved by incubation of the racemic Boc-vinylglycine, D,L-(143), with papain in a biphasic medium. In this instance, as the starting material was much less soluble in the buffer than was the aminoadipate derivative, the ratio of organic phase to aqueous phase was altered from 50 : 1.5 to 3 : 15 . The alcohol chosen was ethanol which could be easily removed under reduced pressure thereby avoiding rigorous purification. The desired Boc-D-vinylglycine, D-(143), was thus simply extracted and recovered from the organic phase by washing with sodium bicarbonate and recovered by re-extraction (Scheme 3.7).

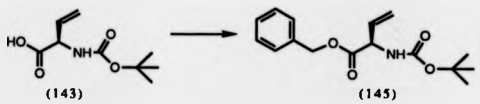


Conditions : Papain, L-cysteine, EDTA Na_4,
citrate / phosphate buffer, pH 4.2,
dichloromethane, ethanol, 37°C, 2 days.

Scheme 3.7

The benzyl ester of the Boc-D-vinylglycine (145) was prepared in high yield (80%) by reaction with phenyl diazomethane (Scheme 3.8)[100]. In contrast to the extreme reactivity of diazomethane, in this instance prolonged stirring and heating to reflux failed to induce the reaction but addition of a small portion of ethereal hydrogen chloride caused the reaction to go immediately to completion. The

product was purified by flash chromatography with ethyl acetate / petroleum ether 40-60° 1 : 10.

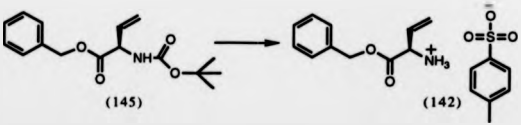


(143) (145)

Conditions : Phenyldiazomethane, diethyl ether, cat. hydrogen chloride, RT.

Scheme 3.8

The extremely sensitive benzyl vinylglycinate *p*-toluenesulphonic acid salt (142) was prepared by stirring with exactly one equivalent of *p*-toluenesulphonic acid in diethyl ether for 4 hours at room temperature[101]. The product was recovered by evaporation under reduced pressure and used without further purification (Scheme 3.9). The proton nmr of the product showed a complete absence of the prominent *t*-butyl signal normally at 1.4 ppm.



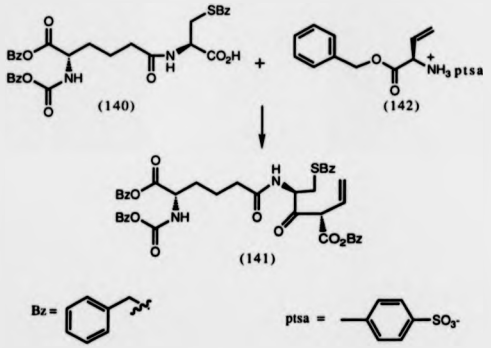
(145) (142)

Conditions : *p*-toluenesuphonic acid, diethyl ether, 4hr, RT.

Scheme 3.9

3.7 : Preparation of benzyl-protected aminoadipylcysteinyl vinylglycine (141).

The fully protected tripeptide was prepared by the EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) mediated coupling of the dipeptide (140) and the vinylglycine derivative (142). The yield of the reaction was only 10% because the conditions had to be sufficiently basic to keep the amino group available for the coupling. However the basicity also resulted in the double bond migrating into conjugation with the ester group with consequent loss of yield. The product was purified by reverse phase HPLC (Scheme 3.10).



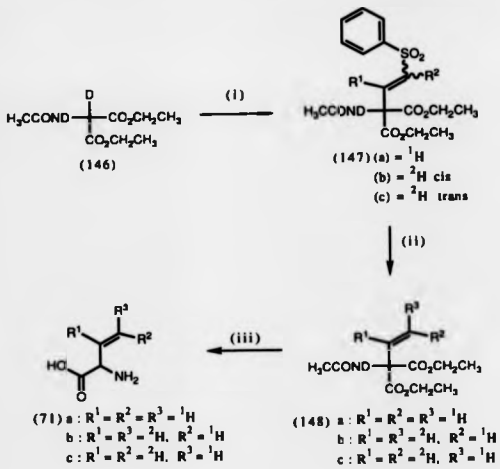
Conditions : EEDQ, THF, triethylamine, RT, 8hr.

Scheme 3.10

Increasing the amount of vinylglycine derivative (142) relative to the dipeptide (140), and the use of the alternative coupling reagent dicyclohexylcarbodiimide failed to increase the amount of tripeptide (141) recovered. The attempted coupling of the free D-vinylglycine with isobutyl chloroformate also failed to give any tripeptide.

3.8 : Preparation of specifically labelled vinylglycine.

A convenient method for the preparation of such labelled vinylglycines was published by Hill *et al*[62] starting from diethyl acetamidomalonate (Scheme 3.11).



Conditions : (i) Phenyl 2-(trimethylsilyl)ethynyl sulphone, THF, D_2O.
(ii) Hg / alumium amalgam, dioxane, 10°C.
(iii) methanol / 6 M HCl, reflux, 4hr.

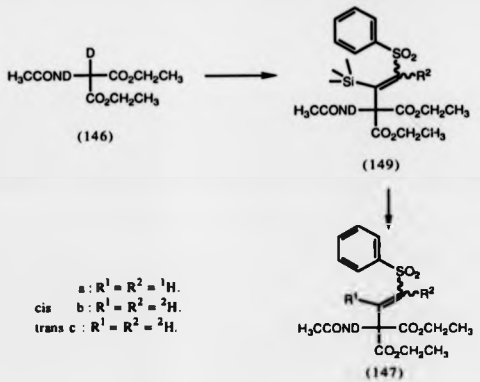
Scheme 3.11

Several problems were encountered whilst attempting to repeat this apparently straight forward procedure.

3.8.1 : Preparation of diethyl acetamido [2-(phenylsulphonyl)-vinyl] malonate (147).

The *Z* and *E*- specifically deuterated vinyl sulphones (147b and c) were prepared by the following method. The labile protons of diethyl

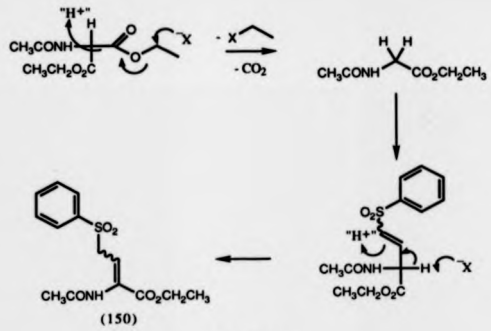
acetamidomalonate (146) were exchanged by twice dissolving the malonate in a mixture of d_1-methanol / deuterium oxide / dry pyridine and evaporating. The malonate (146) so treated showed no signals for the methine or amido protons in the proton nmr spectrum. The d_2 malonate (146) and 0.15 equivalents of freshly sublimed potassium *t*-butoxide were stirred at -10°C in THF before the addition of the phenyl (2-trimethylsilylethynyl)sulphone. The silyl intermediate (149) resulted from the Michael addition of the malonate to the ethynylsulphone. The timing of the addition of the deuterium oxide fixed the ratio of *cis / trans* products formed on removal of the trimethylsilyl group. The kinetic ratio of 3:1 cis sulphone (147b) to the trans sulphone (147c) resulted when the D_2O was added after 1 hour, whereas if it was added after 24 hours the trans sulphone (147c) was recovered almost exclusively as the thermodynamic product (Scheme 3.12). The products were both purified and separated by flash chromatography. Examination of the proton nmr spectra of the individual products showed <5% residual proton.



Conditions : phenyl 2-(trimethylsilyl)ethynyl sulphone, potassium *t*-butoxide, THF, D_2O.

Scheme 3.12

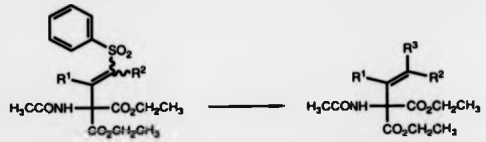
In practice, and contrary to the published procedure, it was found that the time period before addition of the ethynyl sulphone had to be decreased from one hour to thirty minutes to reduce the formation of an unwanted by-product which dramatically affected the yield of the desired products. The by-product was identified as the unsaturated amino acid derivative (150). This compound was possibly formed by a Krapcho-type decarbethoxylation (Scheme 3.13).



Scheme 3.13

3.8.2 : Synthesis of diethyl acetaminovinylmalonate (148).

The de-phenylsulphonation was carried out by stirring the separated *trans* and *cis* isomers of the phenylsulphone (147a and 147b) with aluminium amalgam in 1,4-dioxane for 2 days. At the stated temperature of 5-10°C the yield of product (148a and 148b) was very low (<10%). To increase the yield in the de-phenylsulphonation the temperature of the reaction was increased to room temperature which increased the yield of product to 80% after vacuum distillation (Scheme 3.14).



(147) a : $R^1 = R^2 = {}^1H$.
cis b : $R^1 = R^2 = {}^2H$.
trans c : $R^1 = R^2 = {}^2H$.

(148) a : $R^1 = R^2 = R^3 = {}^1H$.
b : $R^1 = R^3 = {}^2H$, $R^2 = {}^1H$.
c : $R^1 = R^2 = {}^2H$, $R^3 = {}^1H$.

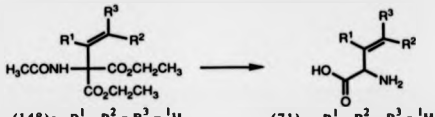
Conditions : Hg / alumium amalgam, dioxane, RT.

Scheme 3.14

Examination of the proton nmr spectra of the purified products showed the expected signal integrating for a single proton.

3.8.3 : Synthesis of deuterated vinylglycine.

The literature method of deprotection of the vinyl malonates (148) was given as boiling under reflux in equal portions of 6 M hydrochloric acid and methanol for four hours. However when this was attempted, little product was recovered. The desired products were recovered in very high yield by boiling the malonates under reflux in 6 M hydrochloric acid for 60 minutes. The solution was then passed down a Dowex 50-8X (H^+) column and the vinylglycine eluted with 4% aqueous pyridine (Scheme 3.15).



(148)a : $R^1 = R^2 = R^3 = {}^1H$
b : $R^1 = R^3 = {}^2H$, $R^2 = {}^1H$
c : $R^1 = R^2 = {}^2H$, $R^3 = {}^1H$

(71) a : $R^1 = R^2 = R^3 = {}^1H$
b : $R^1 = R^3 = {}^2H$, $R^2 = {}^1H$
c : $R^1 = R^2 = {}^2H$, $R^3 = {}^1H$

Conditions : 6 M HCl, reflux , 1hr.

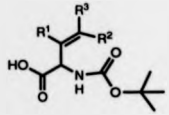
Scheme 3.15

3.8.4 : Labelled D-*t*-butyloxycarbonyl-vinylglycine.

The Boc-vinylglycine derivative was prepared as before in high yield. As for the unlabelled vinylglycine, racemic Boc-vinylglycine was incubated with papain in a biphasic medium to remove the unwanted L-isomer.

The published procedure states that the method is suitable for the preparation of specifically labelled *cis* and *trans* isomers of [3,4-$^2H_2$]vinylglycine. However, no indication was given of the degree of scrambling of the deuterium in the terminal position. The proton nmr spectrum of the labelled Boc-vinylglycine (143) was such that the signals corresponding to both terminal vinylic protons were well resolved as broad singlets. The integrals of these signals permitted the estimation of the % residual proton at both C-4 terminal positions (Table 3.1).

Table 3.1 : Randomisation of deuterium labelling in the terminal position of vinylglycine.



143 b : $R^1 = R^3 = {}^2H$, $R^2 = {}^1H$
c : $R^1 = R^2 = {}^2H$, $R^3 = {}^1H$

Boc vinylglycine	R^2	R^3
143b	2.6	1.0
143c	1.0	2.0

This result showed that randomisation of the deuterium had occurred during the synthesis of the Boc-vinylglycine. The scrambling of the deuterium presumably occurred during the reductive removal of the sulphone substituent. The de-phenylsulphonation of alkenes with sodium amalgam is reported as involving a free radical mechanism which can lead to the scrambling of any labelling[102,103]. This presumably also happens with aluminium amalgam.

The success of the project depended on the specific labelling of the deuterium in the vinylglycine residue. The extent of the randomisation of the label was so great such that the aim of the project could not be fulfilled using labelled tripeptide precursor prepared by the Sawada method.

A final attempt to de-protect the vinylsulphone was made using a biphasic method of Julia *et al*[104]. This employed sodium dithionate and a

phase transfer catalyst in the de-phenylsuphonation. This is reported to proceed through an anion rather than the free radical. However no product was recovered from the reaction mixture corresponding to the starting material, product, or vinylglycine itself.

3.9 : Summary.

(1) As a result of unforeseen scrambling of the labelling in the preparation of the specifically labelled precursor the project could not be completed as planned.

(2) A convenient enzymatic method for the preparation of 1-benzyl α-(*N*-benzyloxycarbonyl)aminoadipate, an important intermediate in the synthesis of a wide range of suitable unnatural substrates for IPNS, using papain has been developed.

CHAPTER FOUR

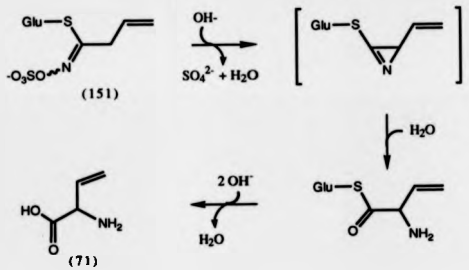
PREPARATION OF VINYLGLYCINE AND OTHER β,γ-UNSATURATED α-AMINO ACIDS BY THE NEBER REARRANGEMENT.

4.1 : Project objective.

It was the need for vinylglycine in the preparation of the unsaturated tripeptide for the experiment on IPNS that prompted our interest in a quick, cheap and efficient synthesis. As indicated in the introduction most of the published methods are relatively long and time consuming with low overall yield. The purchasing of the material from commercial suppliers is prohibitive due to the extremely high cost (£7.40 / mg racemate, £10.10 / mg L-isomer; the D-isomer is not available)[105]. Thus if an inexpensive route to the preparation of vinylglycine could be devised it would allow freer investigation into its uses as an enzyme inhibitor and as a springboard to the preparation of a wide range of other unnatural amino acids by exploitation of the double bond.

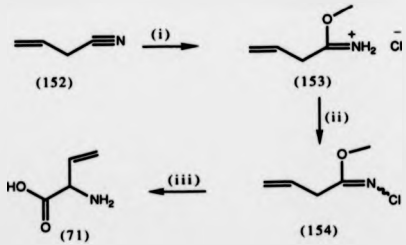
4.2 : General strategy.

The use of the Neber rearrangement in the preparation of amino acids has long been established[107]. In 1977 Friis *et al* reported the formation of vinylglycine (71) upon simple aqueous base treatment of the natural product potassium allylglucosinolate (sinigrin) (151)[106]. This was reported as resulting from a Neber type rearrangement (Scheme 4.1).



Scheme 4.1

It was thus recognised that a synthesis of vinylglycine could be achieved starting from the readily available vinylcyanide. The following pathway was proposed for the preparation (Scheme 4.2). The key step of the synthesis involves a Neber rearrangement of an *N*-chloroimidate to the unsaturated amino acid.



Conditions : (i) Methanol, hydrogen chloride, 0°C, 1 hr.
(ii) Sodium hypochlorite solution, 0°C, 1 hr.
(iii) Sodium hydroxide, 4°C, 8 hr.

Scheme 4.2

4.2.1 : Synthesis of imidate hydrochloride (153).

The preparation of the imidate hydrochloride (153) involved a Pinner acid methanolysis of the nitrile group of vinylcyanide (152) (Scheme 4.3).

(152) (153)

Conditions : Methanol, Hydrogen chloride, 0°C, 1hr.

Scheme 4.3

Hydrogen chloride was passed through a methanolic solution of vinylcyanide for 1 hour at 0°C[107]. The protonation of the nitrile is followed by attack of the methanol. The product of the reaction was a white solid precipitated by the addition of cold, dry diethyl ether. The hygroscopicity of the product disappeared once all traces of the hydrogen chloride were removed. The yield of the reaction was quantitative provided it was carried out at the stated temperature. Control of the temperature during the reaction was essential to prevent the formation of ammonium chloride (if ammonium chloride was formed it would be converted in the next step (sodium hypochlorite treatment) into explosive nitrogen trichloride).

4.2.2 : Synthesis of *N*-chloroimidate (154).

The addition of the sodium hypochlorite solution to an aqueous solution of the imidate hydrochloride (153) typically gave low yields of around 15%. This was a result of hydrolysis of the imidate hydrochloride

to the corresponding amide[108,109]. This hydrolysis was easily followed by observing the proton nmr spectrum in D_2O. After 50 minutes the imidate hydrochloride was completely decomposed. However the addition of solid imidate hydrochloride directly to the hypochlorite solution did not allow time for this hydrolysis and results in the quantitative formation of the volatile, colourless and water insoluble oil which was extracted with petroleum ether (Scheme 4.4).

(153) (154)

Conditions : 12-14% Sodium hypochlorite, 0°C, 1hr.

Scheme 4.4

The *N*-chloro imidate (154) prepared in this manner was highly pure and was used in the next stage without further purification.

4.2.3 : Synthesis of vinylglycine (71).

As stated in the introduction the Neber rearrangement is normally brought about using an alkoxide as a base. However, as with sinigrin, the acidity of the α-proton of the *N*-chloroimidate is again increased by the allylic double bond to the extent that it is eliminated by aqueous alkali (Scheme 4.5)[110].

to the corresponding amide[108,109]. This hydrolysis was easily followed by observing the proton nmr spectrum in D_2O. After 50 minutes the imidate hydrochloride was completely decomposed. However the addition of solid imidate hydrochloride directly to the hypochlorite solution did not allow time for this hydrolysis and results in the quantitative formation of the volatile, colourless and water insoluble oil which was extracted with petroleum ether (Scheme 4.4).

(153) (154)

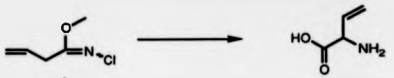
Conditions : 12-14% Sodium hypochlorite, 0°C, 1hr.

Scheme 4.4

The *N*-chloro imidate (154) prepared in this manner was highly pure and was used in the next stage without further purification.

4.2.3 : Synthesis of vinylglycine (71).

As stated in the introduction the Neber rearrangement is normally brought about using an alkoxide as a base. However, as with sinigrin, the acidity of the α-proton of the *N*-chloroimidate is again increased by the allylic double bond to the extent that it is eliminated by aqueous alkali (Scheme 4.5)[110].



(154) (71)

Conditions : 3 eq. sodium hydroxide, 0°C, overnight.

Scheme 4.5

Thus simple base treatment of the oily *N*-chloroimidate with three equivalents of sodium hydroxide overnight at 4°C caused the 1,2 nitrogen migration to occur. The product was isolated by passing the reaction mixture directly over a Dowex 50W 8X (H^+) ion exchange column and eluting with 4% aqueous pyridine.

Examination of the crude product before the ion exchange treatment showed no trace of impurities. However the yield of isolated product after ion exchange was, at best, only 52%. If the column was allowed to stand for a short period of time before continuing the elution a yellow polymeric gum formed. This by-product would appear to result from some polymerisation of the product in the reaction solution on the column. This side reaction was not seen when vinylglycine, from other sources, was passed down the column the recovery was quantitative.

4.2.4 : Synthesis of *N*-*t*-butyloxycarbonylvinylglycine (143).

It was considered that protection of the amino group would make it possible to isolate the protected vinylglycine through extraction. The need for the ion exchange procedure with its attendant side reaction would thus be avoided. Therefore an equal volume of 1,4-dioxane and 1.2 equivalents of di-*t*-butyldicarbonate were added directly to the

(154) (71)

Conditions : 3 eq. sodium hydroxide, 0°C, overnight.

Scheme 4.5

Thus simple base treatment of the oily *N*-chloroimidate with three equivalents of sodium hydroxide overnight at 4°C caused the 1,2 nitrogen migration to occur. The product was isolated by passing the reaction mixture directly over a Dowex 50W 8X (H^+) ion exchange column and eluting with 4% aqueous pyridine.

Examination of the crude product before the ion exchange treatment showed no trace of impurities. However the yield of isolated product after ion exchange was, at best, only 52%. If the column was allowed to stand for a short period of time before continuing the elution a yellow polymeric gum formed. This by-product would appear to result from some polymerisation of the product in the reaction solution on the column. This side reaction was not seen when vinylglycine, from other sources, was passed down the column the recovery was quantitative.

4.2.4 : Synthesis of *N*-*t*-butyloxycarbonylvinylglycine (143).

It was considered that protection of the amino group would make it possible to isolate the protected vinylglycine through extraction. The need for the ion exchange procedure with its attendant side reaction would thus be avoided. Therefore an equal volume of 1,4-dioxane and 1.2 equivalents of di-*t*-butyldicarbonate were added directly to the

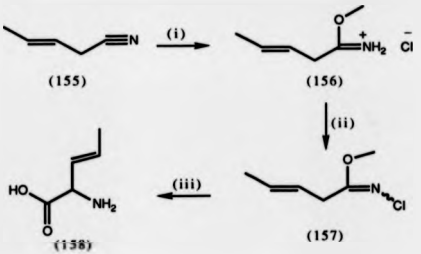
reaction medium at 0°C and the mixture was allowed to stir overnight to prepare *N*-*t*-butyloxycarbonylvinylglycine (143). Careful extraction of the product with ice-cold 1 M potassium hydrogen sulphate increased the yield of the vinylglycine to 67%. Care was required to prevent the de-protection of the acid sensitive *N*-*t*-butyloxycarbonyl group.

4.3 : Synthesis of analogues of vinylglycine.

Having thus established a new method for the preparation of vinylglycine we then examined the feasibility of the method for the preparation of other β,γ-unsaturated amino acids.

4.3.1 : Synthesis of 2-amino-pent-3-enoic acid (158).

Distilled trans pent-3-enenitrile (155), a commercially available material, was subjected to the Pinner reaction and subsequent sodium hypochlorite treatment as above. The yields for these steps was 87% and 94% respectively.



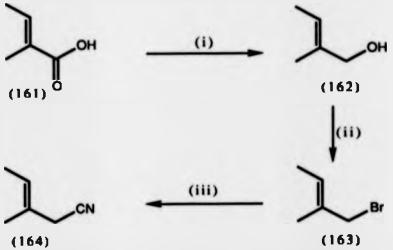
Conditions : (i) Methanol, hydrogen chloride, 0°C, 1 hr.
(ii) Sodium hypochlorite solution, 0°C, 1 hr.
(iii) Sodium hydroxide, 4°C, 8 hr.

Scheme 4.6

The Neber rearrangement in aqueous medium gave a disappointing yield of only 14% β,γ-unsaturated amino acid after ion exchange treatment. The use of the more typical Neber base such as alkoxide had previously been used by Crout *et al*[107] in the preparation of 2-amino-3-methyl-but-3-enoic acid. However use of alkoxide as base gave a similar yield of 12%. The product was not sensitive to the basicity of the solution. A solution of 2-amino pent-3-enoic acid in 0.65 M NaOD solution and heated to 50°C overnight showed no decomposition by proton nmr. Again when the Boc-derivative (159) was prepared, obviating the need for an ion exchange column, as with vinylglycine, an increase in the yield to 29% was observed.

4.3.2 : Synthesis of 2-amino-3-methyl-pent-3-enoic acid (167).

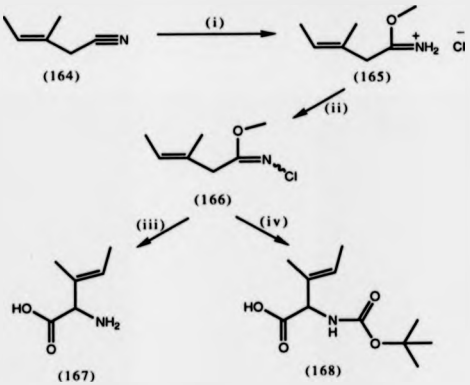
The required nitrile (164), not available commercially, was prepared from methyl tiglate in the following manner.



Conditions : (i) Lithium aluminium hydride, diethyl ether, 0°C, 2 hr.
(ii) Phosphorous tribromide, diethyl ether, 0°C, 8 hr.
(iii) Sodium cyanide, DMSO, 50°C, 2 hr.

Scheme 4.7

Lithium aluminium hydride treatment of an ethereal solution of methyl tiglate at 0°C afforded the corresponding alcohol (162) in 89% yield. Addition of phosphorous tribromide to an ethereal solution of the 2-methyl-but-2-ene-1-ol at 0°C followed by stirring for 8 hours gave the corresponding bromide (163) in 76% yield. The addition of 1-bromo-2-methyl-but-2-ene to a DMSO solution of sodium cyanide at 50°C followed by stirring for 2 hours gave the nitrile (164) in 67% yield (Scheme 4.7).



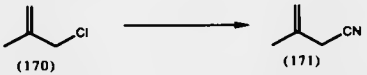
Conditions : (i) Methanol, hydrogen chloride, 0°C, 1 hr.
(ii) Sodium hypochlorite solution, 0°C, 1 hr.
(iii) Sodium methoxide, 4°C, 8 hr.
(iv) Sodium methoxide, methanol, di-*t*-butyl-di-carbonate

Scheme 4.8

Both the Pinner reaction and the bleach treatment proceeded in quantitative yields. The Neber reaction with ethoxide as the base gave a yield of 58% after passing down a ion exchange column. However if the ethoxide solution was quenched into water and reacted with di-*t*-butyl-dicarbonate, a yield of 50% of *N*-*t*-butyloxycarbonyl-2-amino-3-methylpent-3-enoic acid (168) was obtained (Scheme 4.8).

4.3.3 : Synthesis of 2-amino-3-methyl-but-3-enoic acid (173).

3-Methylbut-3-enenitrile (171) was prepared by the slow addition of methallyl chloride to a solution of cuprous cyanide in nitrobenzene heated at 125°C for 2 hours. The product was isolated by fractional distillation in 77% yield[111].



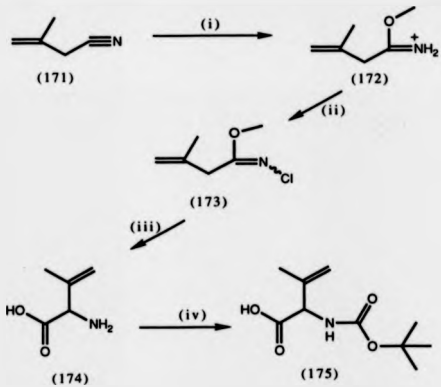
(170) (171)

Conditions : Sodium cyanide, nitrobenzene, DMSO, 125°C, 2 hr.

Scheme 4.9

The usual synthesis by reacting the halide with sodium cyanide in dimethylsulphoxide resulted in the isomerisation of the double bond to give the conjugated 3-methyl-2-butenenitrile as the only product.

The Pinner reaction and subsequent sodium hypochlorite treatment of the nitrile (171) gave yields of 100 and 87% respectively. The use of aqueous base for the Neber rearrangement of *N*-chloroimidate (173) gave a yield of 19% of recovered amino acid (174). The Neber rearrangement gave a yield of 76% with ethoxide as the base[107]. The Boc-derivative was prepared in high yield (100%) with di-*t*-butyl-dicarbonate (Scheme 4.10).

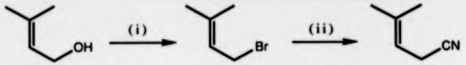


Conditions : (i) Methanol, hydrogen chloride, 0°C, 1 hr.
(ii) Sodium hypochlorite solution, 0°C, 1 hr.
(iii) Sodium ethoxide, 4°C, 8 hr.
(iv) Di-*t*-butyl-dicarbonate, 1,4-dioxane, water, sodium bicarbonate, 0°C, 8 hr.

Scheme 4.10

4.3.4 : Pinner reaction of 4-methyl pent-3-enenitrile (176).

4-Methylpent-3-enenitrile (178) is not available commercially and was prepared from the inexpensive 3-methylbut-3-en-1-ol (176) (Scheme 4.11).



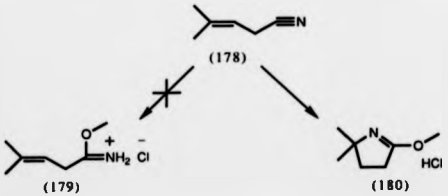
(176) (177) (178)

Conditions : (i) Phosphorous tribromide, diethyl ether, 0°C, 8 hr.
(ii) Sodium cyanide, DMSO, 50°C, 2 hr.

Scheme 4.11

The corresponding bromide (177) was prepared by adding the alcohol to an ethereal solution of 0.33 equivalents of phosphorous tribromide at 0°C in 83% yield. The nitrile (178) was then prepared in 74% yield by stirring the bromide (177) in a solution of 1.2 equivalents of sodium cyanide in DMSO at room temperature overnight.

The Pinner reaction of 4-methylpent-3-enenitrile (178) did not give the expected imidate hydrochloride product (179). The proton nmr gave none of the expected signals. There was a complete absence of the signal due to vinylic protons expected at 5.5-6.5 ppm (Scheme 4.12).

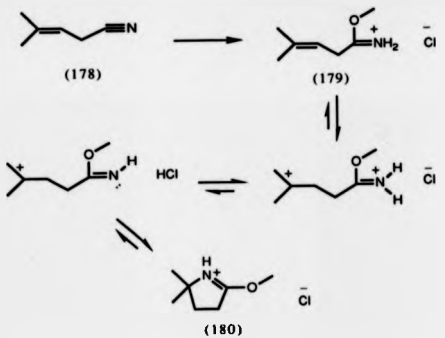


Conditions : methanol, hydrogen chloride 0°C, 1hr.

Scheme 4.12

The analytical data suggested that the structure of the product is the hydrochloride of 5,5'-dimethyl-2-methoxy-1-pyrrolidine (180). The proton nmr spectrum (Figure 1, p92) contains two singlets at 3.64 and 1.52 ppm corresponding to the methoxy and the two methyl protons respectively. The methylene protons displayed a typical A_2B_2 splitting pattern. The splitting pattern of the two signals are identical and symmetrical as the difference of the chemical shifts is large compared to the coupling constants.

The mass spectra and ^{13}C nmr also agree with the proposed structure. This compound would not normally be expected to form under the acidic conditions of the Pinner reaction. A possible mechanism for the formation of the pyrrolidine ring results from the protonation of the double bond of the imidate hydrochloride (179) to give a relatively stable tertiary carbocation (Scheme 4.13). Although the reaction medium is extremely acidic, an equilibrium must exist between protonated and unprotonated imidate species. The lone pair of the neutral unprotonated nitrogen instigates a nucleophilic attack at the carbocation forming an energetically favourable five membered ring (Scheme 4.13). This cyclic structure (180) is of interest for its potential in the preparation of γ-amino acids.

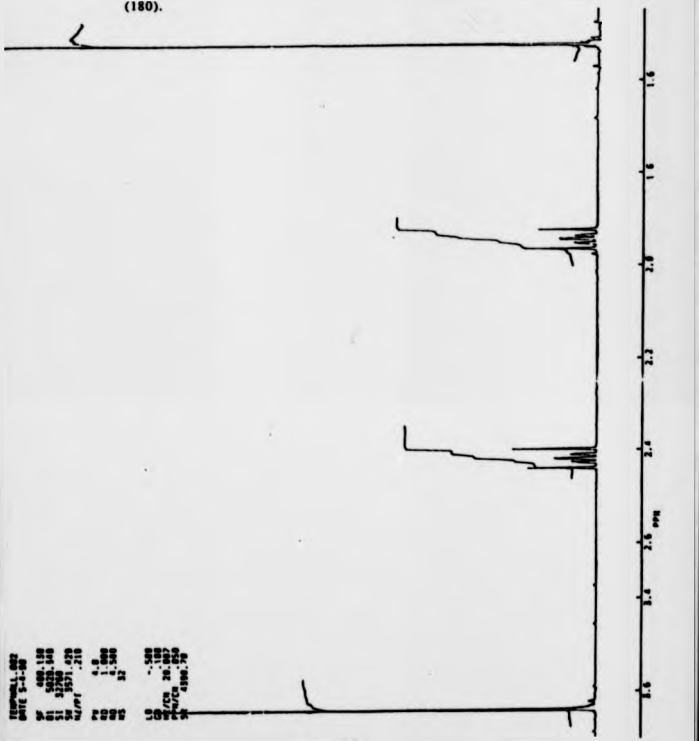


Scheme 4.13

4-Phenyl-but-3-enenitrile and 1,4-but-2-ene dicyanide were subjected to the Pinner reaction and sodium hypochorite treatment successfully but failed to give any product from the Neber rearrangement.

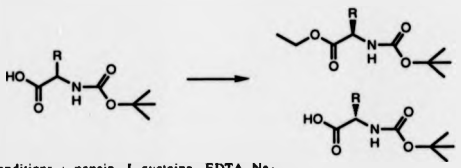
Figure 1 :

^1H nmr of 5,5'-dimethyl-2-methoxy-1-pyrrolidinium hydrochloride (180).



4.4 : Resolution of the unsaturated amino acids Boc derivatives.

Having prepared *N*-*t*-butyloxycarbonyl-amino acids an attempt was made to resolve them into their optical isomers. The simplest way to do this is by enzymic resolution (Scheme 4.14).



Conditions : papain, L-cysteine, EDTA Na_4,
citrate / phosphate buffer, pH 4.2,
dichloromethane, ethanol, 37°C, 2 days.

Scheme 4.14

Papain had previously been used Cantacuzene et al in the resolution of benzyloxycarbonyl-amino acid derivatives[96] and by us in the selective α-benzylation of *N*-benzyloxycarbonyl-2-aminoadipic acid (Chapter 2). Papain is enantiospecific for the the L-isomer of amino acids so this method readily lent itself to the inexpensive resolution of the *N*-*t*-butyloxycarbonyl-amino acids. The conditions of the enzymatic resolution of the amino acids is the same as that given in chapter two. The L-ester (144) was purified by flash chromatography with ethyl acetate / petroleum ether 10 : 1.

A single incubation with papain was not sufficient for the complete resolution of racemic Boc-vinylglycine. To complete the resolution therefore a second incubation with papain of the recovered

impure D-acid was carried out. The optical purity was checked by comparison of the optical rotation of the free amino acids which obtained after the acid hydrolysis and ion exchange treatment.

By this method vinylglycine and 2-aminopent-3-enoic acid were resolved into their L- and D-isomers. The resolution of 2-amino-3-methylpent-3-enoic acid was still incomplete after two incubations. The Boc derivative of 2-amino-3-methylbut-3-enoic acid (173) failed completely to act as a substrate for papain. The unsuitability of 2-amino-3-methyl-but-3-enoic acid to act as a substrate is consistent with the results of Cantacuzene *et al*[96] who had observed that the structurally similar Boc-L-valine failed as a substrate. This indicates possibly that an isopropyl side chain on the amino acid substrate cannot be accommodated in the active site of the papain, whereas *n*-propyl and isobutyl chains are accepted, although in the latter case only poorly. The results are summarised in Table 4.1.

Table 4.1: Optical rotations of the resolved amino acids.

Amino acid	L-isomer	D-isomer
(71)	+95°	-96°
(158)	+112°	-114°
(167)	+65.4°	

4.5 : Synthesis of vinylglycine epoxide (181).

The presence of the double bond in ethyl-L-*N*-*t*-butyloxycarbonyl-vinylglycinate (144) could be used to provide

access to a wide range of unnatural L-α-amino acids. Epoxidation of the double bond and subsequent nucleophilic ring opening is a versatile method by which a wide range of derivatives can be readily synthesised[112]. The epoxide of vinylglycine was prepared in good yield with *m*-chloroperbenzoic acid in 1.2-fold excess (Scheme 4.14)[113].

(144) (181)

Conditions : *m*-chloroperbenzoic acid, carbon tetrachloride, RT

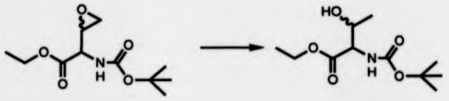
Scheme 4.14

The epoxidation by *m*-chloroperbenzoic acid is by *syn* addition[114]. The chiral centre of the vinylglycine adjacent to the double bond led to asymmetric epoxidation in the ratio of 1 : 4 in chloroform. The signal corresponding to the *t*-butyl protons in the proton nmr indicated the diastereomeric ratio of the product (181). Altering the polarity of the solvent was seen to affect this diastereomeric ratio. Repeating the reaction in carbon tetrachloride increased the diastereomeric ratio to 1 : 6.

The major product was identified as having the *2S,3R* configuration as follows. The Raney nickel catalysed hydrogenolysis of the epoxide product gave the corresponding alcohols (182) (Scheme 4.15)[115]. The tlc of the free amino acids, obtained by acid hydrolysis, against threonine and *allo*threonine, in the solvent system n-butanol /

2-butanone / conc. ammonia / water 15 : 9 : 4 : 2, identified the major diastereomer as threonine.

The nucleophilic ring opening of the epoxide can be used to prepare a large range of unnatural amino acids. Recently Meffre *et al*[116] published a report in which they describe the preparation of a range of β-hydroxy α-amino acids by this route.



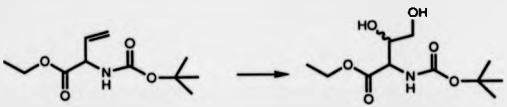
(181) (182)

Conditions : Ethanol, Raney nickel, 10 atm. H_2, 100^{0}C, 8 hr.

Scheme 4.15

4.6 : Synthesis of ethyl *N*-*t*-butyloxycarbonyl-4-hydroxythreonine (183).

Hydroxythreonine is an intermediate in several biochemical pathways[117]. The protected hydroxythreonine (183) was prepared in good yield from ethyl L-*N*-*t*-butyloxycarbonylvinylglycinate (144) using osmium tetraoxide (Scheme 4.16)[118]. The vinylglycine (144) was stirred overnight with osmium tetraoxide at room temperature in an 8 : 1 mixture of *t*-butanol / water to give a diastereomeric mixture of the product diols (183). 4-Methylmorpholine *N*-oxide was used to recycle the osmium tetraoxide (Scheme 4.16)[119].



(144) (183)

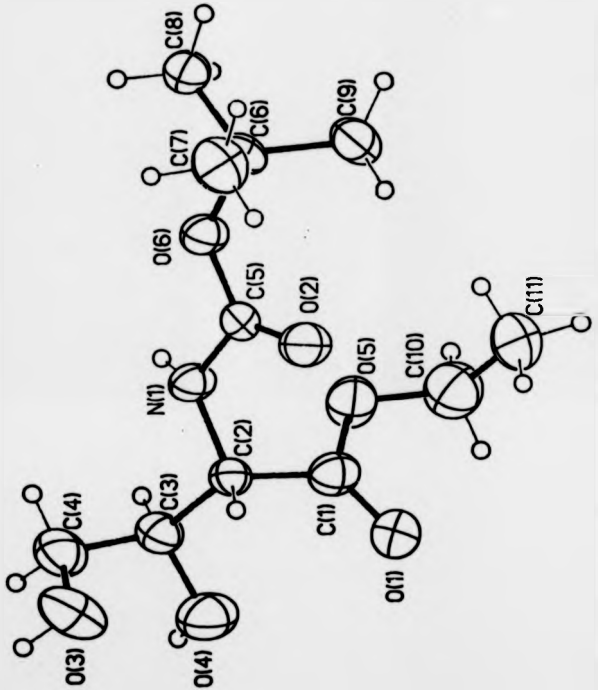
Conditions : *N*-methyl morpholine *N*-oxide, osmium tetraoxide, *t*-butanol, RT

Scheme 4.16

By careful flash chromatography, eluting with 2 : 1 ethyl acetate / petroleum ether, the diastereomers could be separated in a ratio of 2 : 1. The minor component solidified and was recrystallised from ethyl acetate / petroleum ether. Its structure was established by an X-ray diffraction by Doctor W. Errington (Warwick) and it was found to have the 2*S*, 3*R* configuration. As before the hydrolysis of the protected diastereomers in 6 M hydrochloric acid gave the free hydroxythreonine isomers.

Figure 2 :

X-ray structure of ethyl (2*S*,3*R*)-*N*-*t*-butyloxycarbonyl-4-hydroxy threonate (183)



4.7 : Synthesis of ethyl *N*-*t*-butyloxycarbonylcyclopropylglycine (184).

The most common method for the cyclopropanation of double bonds is the Simmons-Smith method involving diiodomethane and zinc. The use of these reagents under a variety of conditions, including heating and sonication[120,121], failed to give any product. However the palladium (II) acetate-catalysed reaction with diazomethane proceeded smoothly to give a product that was purified by flash chromatography with ether / ethyl acetate 10 : 1 as eluent, followed by bulb to bulb distillation to give a colourless oil (184) in 80% yield (Scheme 4.17)[121,122]. The biologically active free amino acid[123] (185) was obtained by hydrolysis with 6 M hydrochloric acid under reflux and ion exchange treatment in 66% yield.

(144) (184)

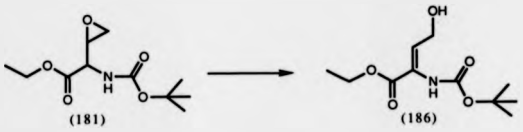
Conditions : diazomethane, palladium (II) acetate, diethyl ether, RT

Scheme 4.17

4.8 : Synthesis of ethyl *N*-*t*-butyloxycarbonyl-2-amino-but-2-enoate (186).

4-Fluorothreonine is a potential enzyme inhibitor. Nucleophilic attack on the epoxide (181) by the fluoride ion could be a simple way to prepare this compound. Stirring potassium fluoride in DMSO or methanol with the epoxide resulted, not in the formation of the desired product,

but in the unsaturated homoserine derivative (186) in 75% yield (Scheme 4.18).



Conditions : potassium fluoride, methanol, RT

Scheme 4.18

This dehydrohomoserine derivative (186) presumably results from the α-deprotonation and elimination (Scheme 4.19).

Scheme 4.19

No trace of the fluorothreonine derivative was detected under these conditions or when the epoxide was stirred with hydrogen fluoride in pyridine[124].

4.9 : Summary.

(1) Vinylglycine and analogues were prepared from an inexpensive starting material in a potentially viable commercial process. The route involves three step synthesis using Pinner reaction, bleach treatment and Neber rearrangement.

(2) The enzyme papain was used to resolve, completely or partially, the Boc-derivatives into their optical isomers.

(3) A range of vinylglycine derivatives were prepared through the reaction of the double bond. These included epoxidation, dihydroxylation and cyclopropanation.

CHAPTER FIVE

THE DEVELOPMENT OF A REAGENT FOR THE DETERMINATION OF ENANTIOMERIC EXCESS OF CHIRAL CARBOXYLIC ACIDS BY NMR.

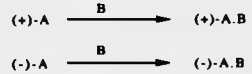
5.1 : Introduction.

The determination of enantiomeric excess (ee) of chiral compounds is of growing importance in all areas of chemistry. Several methods have been developed to determine the enatiomeric excess. The use of chromatographic techniques such as chiral HPLC[125] and GLC[126] has found widespread application. These rely on the different interactions between the optical isomers with the chiral stationary phases, which result in different retention times, thereby permitting determination of the enantiomeric excess.

The use of nmr is another major technique in the determination of ee. Two general approaches have been used. The first relies on the addition of shift reagents to a solution of the chiral compound. The interaction between the shift reagent and the two enantiomers result in the formation of diastereoisomeric complexes with different chemical shifts of the pmr signals, the integrals of which relate directly to the ee of the mixture. Examples of these shift reagents include (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol[127], α-methylamine[128] and chiral lanthanide[129] reagents.

The second approach involves the chemical synthesis of covalent diastereomers of the chiral compound with enantiomerically pure reagents. The resultant pair of diastereomers have two sets of signals in the proton nmr the intensities of which are directly related to the

amounts of the enantiomers present in the original sample (Scheme 5.1). Acetylmandelic acid[131] and α-methoxy-α-(trifluoromethyl)phenyl acetic acid (Mosher's acid)[132] are examples of compounds whose pure enantiomers have found use as such reagents.



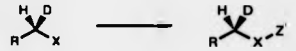
Scheme 5.1

A problem arises in the use of these compounds; namely that the chemical synthesis of the diastereomers must be quantitative. As the products of the reaction are diastereomers, incomplete reaction could lead to a kinetic resolution whereby one diastereomer might be preferentially formed with a rate faster than the other. The purification by crystallisation or chromatography of the diastereomers could also result in the preferential loss of a single diastereomer. If either of these possibilities were to occur then they would give an incorrect estimation of the ee of the chiral compound.

5.2 : Project objective.

The examination of the proton nmr spectra of diastereomers is nevertheless an excellent method for determining ee. The formation of diastereomers with the above reagents always involves the stated risks. The objective of this project was the develop a reagent for the determination of ee where the possibilities for the above kinetic resolution would be reduced to a minimum.

For example, a compound with a prochiral centre, made chiral by isotopic substitution in the attached enantiotopic groups, would give diastereoisotopomeric products when coupled to a racemic or partly racemic compound.



Z^* = chiral entity

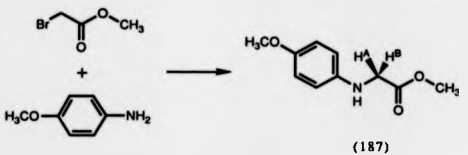
Scheme 5.2

Since the diastereotopicity of the product is due only to the isotopic substitution, there would be no risk of significant kinetic resolution in the coupling step. For the method to work the diastereotopic substituents in the product would have to be distinguishable by nmr. A suitable system for testing this approach would be one based on glycine because the C-2 methylene protons of glycine are enantiotopic. Reacting the glycine with a chiral reagent would result in the formation of diastereotopic methylene protons. The proton nmr spectra of such compounds often demonstrate an AB quartet for the C-2 methylene protons (Scheme 5.3).

Scheme 5.3

5.3 : Preparation of chiral analysis reagent.

To test the above ideas methyl *N*-*p*-methoxyphenylglycinate (187) was prepared by reacting *p*-anisidine with methyl bromoacetate (Scheme 5.4).



(187)

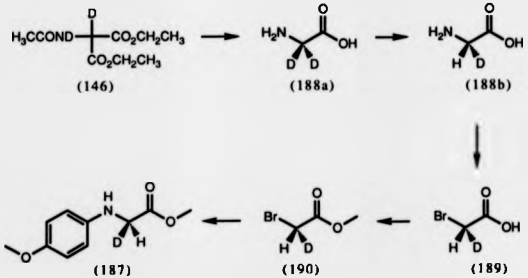
Conditions : Triethylamine, THF, reflux, 2hr.

Scheme 5.4

The *p*-methoxy substituent was necessary to activate the nitrogen of the amine (187) to enable it to react with a range of carboxylic acids. The diastereotopic methylene protons in the product amides would give an AB quartet in the pmr. The intensities of the signals of the doublets would be first order.

Stereospecifically replacing one of the protons by a deuteron in the amine (187) would result in the formation of diastereomers when coupled with a chiral acid. This would result in the formation of a pair of diastereomers in which the signals corresponding to the C-2 proton would be widely separated, as they are in different environments. The signal for the glycinate proton would be expected to appear as a poorly resolved triplet resulting from geminal proton-deuterium coupling (normally in the order of 0-2Hz). The intensity of the signals due to the

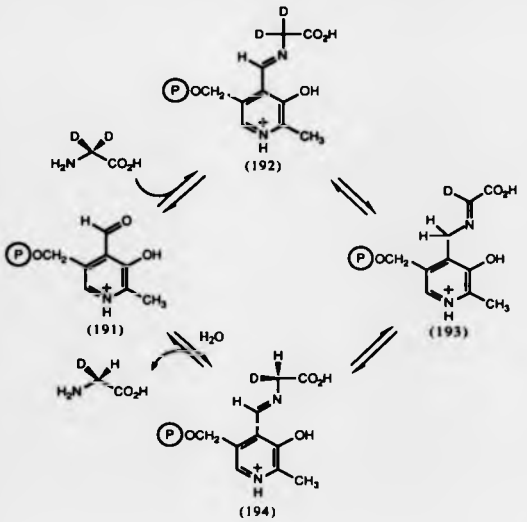
glycinate protons of the two diastereomers could therefore be used as a direct measure of the ee of the optically active acid. As the only difference between the two diastereomers is isotopic, kinetic resolution would be reduced to a minimum and would be expected to be insignificant, as noted above. The chiral reagent (187) was prepared as outlined in Scheme 5.5.



Scheme 5.5

5.3.1 : Preparation of (2S)-[2-$^2H_1$]glycine (188b).

There are many published methods for the preparation of specifically deuterated glycine[132,133,134,135,136,137,138,139]. However, most of the methods involve extremely long synthetic or chemoenzymatic routes. The simplest method, involving the use of glutamic pyruvic transaminase, has been used by Gani *et al*[139] and Arigoni and Beamer[140,141] in their preparation of specifically labelled glycine.

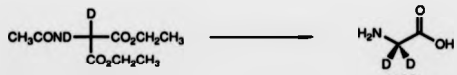


Scheme 5.6

Transaminases catalyse the stereospecific interconversion of L-amino acids and α-ketoacids. In the absence of an α-ketoacid the reaction with glycine can only proceed as far as the Schiff's base. The intermediate is then stereospecifically hydrolysed back to free glycine. Thus when dideuterated glycine is incubated with glutamic pyruvic transaminase and water of normal isotopic composition, stereospecific reprotonation of the intermediate (193) leads, after hydrolysis of the Schiff's base (194), to stereospecifically labelled (S-$^2H_1$)glycine (188b) (Scheme 5.6)[142].

As the reagent is for use with proton nmr it is essential that the presence of the diprotiated species should be avoided. The presence of the diprotiated species would interfere with, and greatly complicate the estimation of the enantiomeric excess. To eliminate this possibility the enzyme was incubated with dideuterated glycine. As the dideuterated species is invisible to proton nmr complete "washing out" of the pro-*R* deuterium is not required though of course as much as possible should be removed to ensure maximum sensitivity.

The method of Upson and Hruby[143] for preparing α-deuterated amino acids, by boiling glycine under reflux with acetic anhydride and d_1-acetic acid, gave very poor results with 9% of residual proton remaining in the acetylglycine after two treatments. The most convenient and reliable method of preparing d_2-glycine is the acid hydrolysis of deuterium exchanged diethyl acetamidomalonate (Scheme 5.7)[144]. The use of twice deuterium exchanged diethyl acetamido malonate gave much better deuterium content in the product than the reported use of unexchanged diethyl acetamidomalonate. The acidic solution of deuterium chloride in deuterium oxide was prepared by adding thionyl chloride to ice cold deuterium oxide. The dideuterated product was isolated by concentrating to dryness and passing over a Dowex 50W-8X(H^+) ion exchange resin as before. The proton nmr of the Boc-d_2-glycine derivative showed only the *t*-butyl protons.



(146) (188)

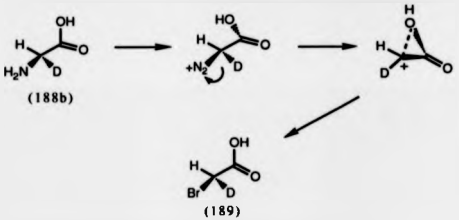
Conditions : DCl / D_2O

Scheme 5.7

The incubation of d_2-glycine with the glutamic pyruvic transaminase leads to the formation of (2*S*)-[2-$^2H_1$]-glycine (starting from $^2H_2$-glycine in H_2O) with 2-*pro*-*R* protonation. Arigoni and Besmer reported that the incubation proceeded at pH 6.6[142]. However, on repetition of their procedure at this pH, no proton incorporation was seen. Proton incorporation occurred at the pH near the optimum for the transaminase namely pH 7.1. The reaction mixture was stirred for two days in the dark at 37°C. For the enzymatic reaction to succeed it was also essential that the pH of the solution was corrected to pH 7.1 after the addition of the pyridyl-phosphate before the addition of the enzyme. The mixture was worked up by passing the solution directly over a column of Dowex 50W-8X (H^+) and eluting the product with 4% aqueous pyridine. The proton nmr spectrum of the Boc-(*S*)-[2-$^2H_1$]-glycine derivative showed signals corresponding to a single C-2 proton and nine *t*-butyl protons.

5.3.2 : Preparation of Methyl (2*S*)-[2-$^2H_1$]bromoacetate (190).

In order to introduce the electron-donating *p*-methoxyphenyl ring into the glycine it was necessary to replace the amino group by bromine which in turn could be displaced with *p*-anisidine. The reaction of sodium nitrite with α-amino acid in an acid solution is reported as proceeding with retention of configuration[145]. This retention is attributable to interaction with the carboxylate group rather than to the formation of an α-lactone. The carboxylate group shields one side of the carbocation from nucleophilic attack, forcing the incoming nucleophile to attack from the same side as the leaving group (Scheme 5.8).



(188b)

(189)

Conditions : sodium nitrite, hydrobromic acid, 0°C

Scheme 5.8

When this procedure was carried out on the monodeuterated glycine the chiral bromoacetic acid thus derived was isolated by repeated extraction with diethyl ether. Bromoacetic acid (189) was methylated by the use of diazomethane. The ether was then carefully distilled from the low boiling monodeuterated methyl bromoacetate (190) which was itself purified by bulb-to-bulb distillation.

Methyl *N*-*p*-methoxyphenylglycinate (both stereospecifically monodeuterated and unlabelled) was prepared by refluxing methyl bromoacetate, *p*-anisidine and 1.2 equivalents of triethylamine in dry THF for two hours. The product was purified by flash chromatography eluting with ethyl acetate / petroleum ether 5 : 1. The product was expected to be methyl N-*p*-methoxyphenyl(2*R*)-[2-$^2H_1$]-glycinate, given that the displacement of the bromine should proceed by an S_N2 mechanism with inversion.

Examination of the mass spectrum of the methyl *N*-*p*-methoxy phenyl(2*R*)-[2-$^2H_1$]-glycinate revealed the isotopic composition of the

product thereby indicating the percentage of di-protiated, mono, and dideuterated species present (Table 5.1).

Table 5.1 : Isotopic composition of chiral reagent 187.

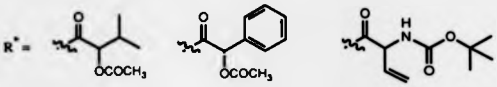
187	$^{1}H_2$	$^{2}H_1$	$^{2}H_2$
ratio	6.9	79.1	14.0

5.4 : Preparation of diastereomers of methyl *N*-*p*-methoxyphenyl (2*R*)-[2-$^{2}H_1$] glycinate with chiral acids.

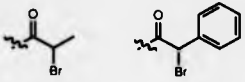
Methyl *N*-*p*-methoxyphenylglycinate (187) was coupled with a range of racemic carboxylic acids using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) to give the amides in high yields. The products were purified by flash chromatography with ethyl acetate / petroleum ether 10 : 1 (Scheme 5.9).

(187)

Conditions : EEDQ, THF, rt, 8hr.



(195) (196) (197)



(198) (199)

(a) : R = H
(b) : R = D

Scheme 5.9

The proton nmr spectra of the diprotiated amides (195-9a) showed the expected AB quartet for the signal corresponding to the diastereotopic glycinate C-2 protons (Table 5.2). The glycinate protons showed a large difference in their chemical shifts and had a large geminal coupling constant of 17 Hz. Reacting racemic carboxylic acids with the monodeuterated glycine derivative likewise gave the expected signals in the proton nmr. In place of the AB quartet two triplets were observed corresponding to a single glycinate proton in the two diastereomers formed (Figure 3).

Figure 3

a : 250 MHz proton nmr of methyl *N*-(4-methoxyphenyl)-*N'*-(*O*-acetyl-mandelate)[2-$^2H_1$]glycinate (196).

b : 250 MHz proton nmr of methylene protons of methyl *N*-(4-methoxyphenyl)-*N'*-(*O*-acetyl-mandelate)glycinate (196)

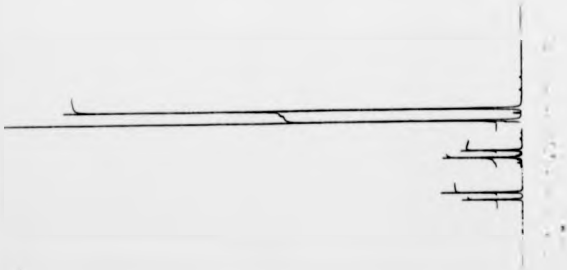


Table 5.2 : chemical shift and J values of diastereomers 195-199.

amide	a			b	
	H^{α}	H^{β}	J (Hz)	H^{α}	H^{β}
195	4.71	3.95	17	4.68	3.98
196	4.41	4.01	17.1	4.50	4.1
197	4.41	4.13	17	4.40	4.15
198	4.51	4.17	17.1	4.49	4.16
199	4.46	4.25	17.1	4.42	4.26

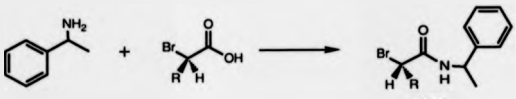
To conclusively prove that these signals were indeed from the glycinate proton of the monodeuterated diastereomers, the monodeuterated reagent was coupled with enantiomerically pure *O*-(+)-acetylmandelic acid. This should give a single triplet corresponding to either the 'downfield' H^{α} or the 'upfield' H^{β} signal. However the proton nmr spectrum of this product was identical to the racemic material. This unexpected result could only be be due to two possibilities :

1. The EEDQ coupling reagent racemised the *O*-(+)-acetylmandelic acid.
2. The monodeuterated glycine reagent was racemised at some stage in its synthetic pathway.

The use of DCC as the coupling reagent in the preparation of the pure diastereomer gave a similar proton nmr spectrum with two triplets. This indicated that the second possibility of the glycine moiety being racemised in its preparation was more likely. The enantiospecificity of the transaminase protonation of glycine is well established[140,141,142]. Of all the steps in the chemical synthesis of the reagent, the bromination

of the chiral glycine was thought to be the most likely cause of racemisation.

To test this possibility the approach of preparing diastereoisotopomeric derivatives was again used. Coupling $^1H_2$-bromoacetic acid with α-methylbenzylamine gave the amide (200a) which also displayed an AB quartet although with strong second order distortion (coupling constant J = 13.8 Hz).



Conditions : EEDQ, THF, rt, 8hr.

(200)

(a) : R = H
(b) : R = D

Scheme 5.10

The presumed optically active bromoacetic acid was initially coupled with racemic α-methylbenzylamine to give a mixture of diastereomers of amides (200b). The proton nmr of this product showed the expected two triplets corresponding to a single proton. The reaction with (*S*)-(-)-α-methylbenylamine gave the amide (200b) the proton nmr spectrum of which also displayed a pair of triplets (Table 5.3).

Table 5.3 : Chemical shift of C-2 protons of amide 200.

Amide	H^{α}	(ratio)	H^{β}	(ratio)
(200a)	3.91		3.90	
(200b)	3.89	(5)	3.86	(4)
(200b)*	3.89	(5)	3.86	(4)

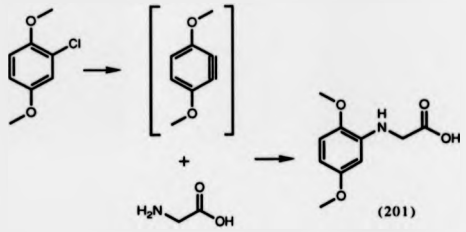
* using (*R*,*S*)-α-methylbenzylamine.

(The intensities of the triplets for the monodeuterated amides (200b) were not equal but had a ratio of 5 : 4. This was a result of a small amount of diprotiated amide present which, as a result of isotopic shift, overlapped with the downfield H^{α} proton. The presence of this unexpected species was revealed by nmr relaxation experiments and by mass spectra of the amide (200b). This problem was not observed in the amides 195-199 because of the proton nmr spectrum was first order. Thus the signal for the C-2 proton in the mono-protiated species was between the signals for the residual diprotiated species.)

This indicated that the monodeuterated bromoacetic acid recovered from the diazotisation of the optically pure monodeuterated glycine had racemised. This had not been expected as in all previous applications of this method for the halogenation of amino acid derivatives the reaction had proceeded with retention. All these cases however used secondary amino acids which reacted *via* secondary carbocations[145,146,147,148]. A possible explanation is that when glycine is treated in this way the primary carbocation which forms is too

reactive for it to be effectively stabilised by the carboxylate group, so that the configurational integrity of the glycine is not maintained.

In order to overcome this problem of racemisation in the bromination step the direct formation of the *N*-*p*-methoxyphenylglycine (201) from glycine by the use of benzynes was investigated (Scheme 5.11)[149]. 1,4-Dimethoxy-2-chlorobenzene was used as the benzyne precursor. This would give a single, highly activated product compared with 1-chloro-4-methoxybenzene which would favour the formation of the less activating meta methoxy isomer over the desired more activating ortho or para derivatives.



Conditions : Potassium, liquid ammonia, iron (III) nitrate, 2hr.

Scheme 5.11

The desired product could not be isolated from the reaction mixture.

5.4 : Summary.

It is clear that the principle of using diastereoisotopomeric derivatives for chiral analysis is a valid one. In the present case, the practical application of the method was vitiated by the unexpected racemisation of the (*S*)-[2-$^{2}H_{1}$]-glycine in the diazotisation step.

CHAPTER SIX.

EXPERIMENTAL DETAILS.

6.1 : Introduction.

Chemicals used in the following syntheses were either purified according to the literature methods or were purchased as the highest available grade.

Nuclear magnetic resonance (nmr) spectra were recorded using the following instruments :

Bruker WH-400 (operating frequency ^{1}H nmr = 400 MHz, ^{13}C = 100 MHz).

Bruker ACF-250 (operating frequency ^{1}H nmr = 250 MHz, ^{13}C = 63 MHz).

Perkin-Elmer R-34 (operating frequency ^{1}H nmr = 220 MHz).

Chemical shifts are given in δ relative to TMS (δ = 0.0ppm).

Multiplicities of the proton nmr are abbriviated as follows:

s, singlet; br. s, broad single; d, doublet; br. d, broad doublet; t, triplet; q, quartet; m, multiplet.

Thin layer chromatography was routinely used to follow the course of reactions on glass precoated with 0.2mm Merck silica gel 60_{F254}.

Methods of detection involved one or more of the following methods :

U.V. fluorescence quenching.

Potassium permanganate (0.5% in water).

Phosphomolybdic acid (7% in ethanol) and heating.

Ninhydrin (0.2% in *n*-butanol) and heating.

Flash chromatography involved columns of 15-20cm of Kieselgel 60 (230-400 mesh ASTM). The solvents used were either glass distilled or of high grade.

Optical rotation were measured on an Optical Activity Limited Model AA-1000 polarimeter. Wavelength = 589nm. Cell length = 2dm. Concentration of samples are given in g / $100cm^3$.

Infra red (IR) spectra were recorded on either Perkin-Elmer 580-B or Perkin-Elmer 1720X instruments.

Mass spectra (MS) were recorded on a Kratos MS 80. X-ray crystal structure on a Nicolet $P2_1$.

6.2 : EXPERIMENTAL DETAILS FOR CHAPTER THREE.

Preparation of (*R*)-2-(*N*-benzyloxycarbonyl)aminoadipic acid (138)[92]

A solution of benzyl chloroformate (0.63g, 2.35mmol) in toluene ($2cm^3$) was added over two hours to a vigorously stirring solution of (*R*)-2-aminoadipic acid (0.25g, 1.5mmol) in water ($1.0cm^3$), 2 M sodium hydroxide ($1.3cm^3$) and acetone ($1.3cm^3$) at 0°C. After the addition was completed the solution was stirred for an extra 30 minutes at room temperature. The pH of the solution was maintained in the region of 8.0-8.7 throughout the reaction by the addition of further 2 M NaOH. Water ($10cm^3$) was added to the mixture and the organic phase removed. The aqueous phase was washed with diethyl ether ($2x10cm^3$) to remove residual benzylchloroformate. The aqueous layer was acidified to congo red with 5 M HCl and extracted with diethyl ether ($2x10cm^3$). The

combined ethereal layers were washed with water ($10cm^3$), saturated brine ($10cm^3$), dried over magnesium sulphate and evaporated under reduced pressure. The white solid product was recrystallised from ethyl acetate / petroleum ether to give the title compound (138) (0.37g, 83%). M. p. 134-135°C. $[\alpha]_D$ = -9.29° (CH_3OH, 22.4°C, c=1.07). δ_H (220 MHz; $CDCl_3$) 7.45 (5H, br. s., Ph-*H*), 5.15 (2H, s, Ph-CH_2), 4.2 (1H, m, *CH*), 2.35 (2H, t, *J* 7 Hz, $OCCH_2$), 1.8 (4H, m, CH_2). m/z (EI) 295 (M^+, 32%), 284 (10), 264 (11), 250 (38), 236 (26), 206 (100), 185 (15.5), m/z (CI) 296 ($M+1^+$, 21%), 252 (38), 234 (11), 205 (100), 187 (3). $\nu_{max}(CHCl_3)/cm^{-1}$ 2928, 1712, 1496. Agrees with literature data[92].

Preparation of α-benzyl *N*-benzyloxycarbonyl-L-2-aminoadipic acid (139)[92].

In a citrate / phosphate buffer solution (1 M, pH 4.2, $3cm^3$), papain (ex-Sigma, type 2 crude, 0.13g), L-cysteine (18mg) and ethylenediaminetetraacetic acid tetra sodium salt (1 M, 30μL) were allowed to stir for 10 minutes. *N*-benzyloxycarbonyl-L-2-aminoadipic acid (0.14g, 0.5mmol) in dichloromethane ($50cm^3$) and benzyl alcohol ($1.5cm^3$) were added and the solution was stirred at 37°C for 2 days. The reaction mix was filtered through a bed of celite and the cake washed with dichloromethane ($15cm^3$) and water ($15cm^3$). The organic layer was separated off and the aqueous phase extracted with ethyl acetate ($2x25cm^3$) and acidified with 1 M potassium bisulphate solution to pH 2.0. The combined organic phase was washed with water ($10cm^3$) and saturated brine ($10cm^3$). The solution was dried over magnesium sulphate and concentrated under reduced pressure to give an oil which was flash chromatographed with ethyl acetate / petroleum ether 1 : 20 solvent system to remove the benzyl alcohol. The product was eluted with

ethyl acetate / petroleum ether 2 : 1 to give an oil which solidified and was recrystallised from ethyl acetate / petroleum ether to give the title compound (139) (0.11g, 60%). M. p. 89.5-90°C. $[\alpha]_D$ = -13.8° (H_2O, 24.6°C, c=1.01). δ_H (400 MHz; $CDCl_3$) 9.8 (1H, br. s, COO*H*), 7.45 (10H, br. s, Ph*H*), 5.54 (1H, br. d, J 8.3 Hz, N*H*), 5.15 (2H, br. s, PhCH_2), 5.10 (2H, s, PhCH_2), 4.45 (1H, m, C*H*), 2.35 (2H, m, OCCH_2), 1.8 (4H, m, CH_2). δ_C (100 MHz; $CDCl_3$) 178.1 (C1), 172.0 (C6), 155.9 (C=O Z), 136 (C1 Ph), 135.1 (C1 Ph), 128.5 (Ph), 128.4 (Ph), 128.3 (Ph), 128.0 (Ph), 127.9 (Ph), 77.2 (C-Ph), 76.9 (C-Ph), 53.6 (C2), 33.0 (C3), 31.6 (C4), 20.2 (C5). m/z (CI) 385 (M+1$^+$, 21%), 342 (18), 295 ((M-Bz)$^+$, 7), 250 (16), 234 (4), 206 (20), 144 (3.5). ν_{max} ($CHCl_3$)/cm^{-1} 2928, 1712, 1496. Agrees with literature data[92].

Preparation of α-benzyl *N*-benzyloxycarbonyl-L-2-aminoadipyl-*S*-benzyl-cysteine (140)[98].

A solution of α-benzyl *N*-benzyloxycarbonyl-L-2-aminoadipic acid (139) (0.248g, 0.64mmol) and triethylamine (204µl, 0.64mmol) in THF (dry, 7cm^3) under nitrogen and was stirred for 5 minutes at -15°C. *Iso*butyl chloroformate (95µl, 0.62mmol) was added and the reaction mixture allowed to stir for a further 30 minutes. A solution of *S*-benzyl-cysteine (0.132g, 0.62mmol), triethylamine (120µl, 0.34mmol) in water (6cm^3), precooled to 0°C, was added in one portion. It was then stirred for 1 hour at room temperature when it was diluted with water (10cm^3). The resulting solution was washed with diethyl ether (10cm^3), then layered with ethyl acetate (15cm^3) and acidified to congo red with 1 M HCl. The organic phase was separated off and washed with water (10cm^3) and saturated brine (10cm^3), dried over magnesium sulphate and evaporated under reduced pressure to give the title compound (140) which was used without further purification (0.31g, 82%). δ_H (400 MHz; $CDCl_3$) 9.0 (1H, br.

s, COO*H*), 7.45 (15H, br. s, Ph*H*), 6.7 (1H, br. d, J 7.3 Hz, N*H*), 5.54 (1H, br. d, J 8.1 Hz, N*H*), 5.15 (4H, m, PhCH_2), 4.7 (1H, m, C*H*), 4.4 (1H, m, C*H*), 3.67 (2H, s, SCH_2Ph), 2.81 (2H, m, CHCH_2), 2.2 (2H, m, OCCH_2), 1.8 (4H, m, 2xCH_2). δ_C (100 MHz; $CDCl_3$) 173.2, 173.0, 172.1, 155.9 , 137.6 (C1 Ph), 135.1 (C1 Ph), 128.8 (Ph), 128.5 (Ph), 128.4 (Ph), 128.3 (Ph), 128.1 (Ph), 127.9 (Ph), 127.1 (Ph), 67.1, 53.4 (C2), 51.4 (C2), 36.4, 34.9, 33.0, 31.4, 20.2. m/z (EI) 385 (M^+, 28.5%), 488 (10.7), 297 (20.9), 255 (12.3), 221 (11.4), 171 (10.1), 148 (100). ν_{max} ($CHCl_3$)/cm^{-1} 3200b, 1714, 1510. Agrees with literature data[98].

Preparation of benzyl (*R*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-butenoate (145)[100].

A solution of sodium hydroxide (0.215g, 5.3mmol), water (0.5cm^3) and methanol (2.8cm^3) was added to a solution of azibenzil (0.15g, 0.67mmol)[100] in diethyl ether (3.4cm^3) in a loosely stoppered conical flask (25cm^3) and allowed to stand for 8 hours with periodic shaking. The reaction was filtered and the filter cake was washed with diethyl ether (5cm^3). The solution was treated with 10% sodium hydroxide (5cm^3) which caused it to separate into two layers. The upper organic phase was removed and treated with 10% NaOH (4x3cm^3) and dried over magnesium sulphate. This solution was added to a stirred mixture of (*R*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-butenoic acid (75mg, 0.4mmol) in diethyl ether (15cm^3). A drop of ethereal hydrogen chloride was added to catalyse the reaction which proceeded with the concomitant evolution of gas. The solution was washed with sodium bicarbonate (5%, 10cm^3) and saturated brine (10cm^3), dried over magnesium sulphate and concentrated under reduced pressure to give an oily residue which was purified by flash chromatography eluted with ethyl acetate / petroleum ether 1 : 3 to give the title compound (145) (97mg, 88%). δ_H (400 MHz;

$CDCl_3$) 7.33 (5H, m, Ph-*H*), 5.90 (1H, ddd, J 16.5, 10.3, 5.3 Hz, C*H*=CH_2), 5.33 (1H, dd, 17.1, 1.6 Hz, CH=C*H*H trans), 5.21 (1H, dd, J 10.3, 1.5 Hz, CH=CH*H* cis), 5.22, 5.19, 5.17, 5.14 (2H, AB q, J 12.3 Hz, CH_2-Ph), 4.91 (1H, unres. t, C*H*), 1.44 (9H, s, *t*-Bu). δ_C (100 MHz, $CDCl_3$) 170.4 (C1), 154.8 (C=O Boc), 135.1 (Ph-1), 132.5 (C3), 128.3 (Ph-2), 128.3 (Ph-4), 128.0 (Ph-3), 117.4 (C4), 80.0 (C-Me_3 Boc), 67.2 (CH_2Ph), 55.8 (C2), 28.1 (CH_3 Boc). m/z (CI) 292 ($(M+1)^+$, 51.7%), 254 (17.1), 253 (73.0), 236 ($(M+1-C_4H_8)^+$, 75.0), 192 ($(M+1-C_4H_8-CO_2)^+$, 80.0), 156 (52.9), 108 (36.9). m/z (EI) 292 ($(M+1)^+$, 0.2%), 156 (72.1), 100 (88.9), 91 ($C_7H_7^+$, 36.9). ν_{max} ($CHCl_3$)/cm^{-1} 1713, 1680, 1455.

Preparation of benzyl (*R*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-butenoate *p*-toluene sulphonic acid (142)[101].

Benzyl (*R*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-butenoate (97mg, 0.33 mmol) was dissolved in diethyl ether (20cm^3) and stirred at 0°C while *p*-toluenesulphonic acid (1eq., 63.6mg, 0.33mmol) in ethanol (15cm^3) was added over 2 hours. The mixture was stirred overnight. The solvent was removed under reduced pressure to give the title compound (142) that was used without further purification (124.4mg, 100%). δ_H (220 MHz; $CDCl_3$) 7.8 (2H, d, J 8 Hz, α-*H* ptsa), 7.3 (5H, m, Ph-*H*), 7.1 (2H, d, J 8 Hz, β-H_2 ptsa), 5.82 (1H, m (ddd), C*H*=CH_2), 5.4 (1H, d, 16 Hz, CH=C*H*H trans), 5.25 (1H, d, J 12 Hz, CH=CH*H* cis), 5.05 (2H, AB q, J 10 Hz, CH_2-Ph), 4.91 (1H, m, C*H*), 2.3 (3H, s, CH_3 ptsa).

Preparation of α-benzyl-*N*-benzyloxycarbonyl-L-2-aminoadipoyl-*S*-benzyl-L-cysteinyl-D-2-amino-3-butenoic acid benzyl ester (141)[98].

A solution of benzyl (*R*)-2-amino-3-butenoate *p*-toluene sulphonic acid (142) (127mg, 0.33mmol) in dichloromethane (dry, 5cm^3) was added to a solution of α-benzyl *N*-benzyloxycarbonyl-L-2-aminoadipoyl-*S*-benzyl-cysteine (140) (0.188g, 0.33mmol), triethylamine (45μl, 0.33mmol) and EEDQ (1.2eq., 0.097g, 0.4mmol) in dichloromethane (dry, 10cm^3) and the solution allowed to stir overnight at room temperature. The solvent was removed under reduced pressure and the residue taken up in ethyl acetate (15cm^3). The organic layer was washed with sodium bicarbonate (5%, 10cm^3) and saturated brine (10cm^3), dried and concentrated under reduced pressure. The solid product was recrystallised from ethyl acetate / petroleum ether. The product was finally purified on C-18 column with a Waters model 440 HPLC (eluent water 66%, acetonitrile 33%, trifluoroacetic acid 1%) (25mg, 10%). δ_H (400 MHz; $CDCl_3$) 7.33 (20H, m, Ph-*H*), 6.93 (1H, d, J 7.7 Hz, N*H* cys), 6.2 (1H, d, J 7.6 Hz, N*H* vg), 5.90 (1H, ddd, J 17.1, 10.4, 5.4 Hz, C*H*=CH_2 vg), 5.55 (1H, d, J 8.0 Hz, N*H* aa), 5.31 (1H, dd, 17.1, 1.5 Hz, CH=C*H*H trans vg), 5.21 (1H, dd, J 10.4, 1.4 Hz, CH=CH*H* cis vg), 5.1 (6H, m, CH_2Ph), 4.52 (1H, m, C*H*), 4.4 (2H, m, C*H*), 3.73 (2H, s, SCH_2Ph), 2.88, 2.86, 2.85, 2.83, 2.70, 2.69, 2.67, 2.65 (2H, 2xAB q, J 14 Hz, CHCH_2S cys), 2.2-1.6 (6H, m, CH_2 aa). δ_C (100 MHz, $CDCl_3$) 171.9, 169.7, 169.7, 137.9, 136.1, 135.1, 134.9, 131.1, 130.2, 129.0, 128.9, 128.6, 128.5, 128.4, 128.2, 128.04, 128.01, 127.2, 118.23, 77.1, 67.5, 67.1, 67.0, 54.6, 53.5, 51.9, 36.5, 35.2, 32.9, 32.9, 31.6, 31.8, 21.2

Preparation of diethyl acetamido[*Z*- and *E*-2-(phenylsulphonyl)-ethenyl] malonate (147a, b and c)[62].

An anhydrous solution of diethyl acetamidomalonate (146) (3.96g, 18mmol) and potassium *t*-butoxide (freshly sublimed, 0.3g, 1.8mmol) in THF (12cm^3) was stirred for 30 minutes at room temperature under nitrogen. The temperature was lowered to -10°C and phenyl 2-(trimethylsilylethynyl) sulphone (3.0g, 12mmol) in THF (dry, 12cm^3) was added and stirred for one hour at this temperature. After a further hour water (3cm^3) was added and the reaction allowed to stir overnight. The reaction was extracted with diethyl ether (15cm^3). The ethereal layer was washed with dilute NaOH (1 M, 5cm^3) to remove excess diethyl acetamidomalonate. The organic layer was dried first over magnesium sulphate and finally over 3A molecular sieves before being evaporated under reduced pressure to give an oily residue. Flash chromatography using chloroform / diethyl ether / petroleum ether 1 : 1 : 3 separated the *Z*- and *E*-isomers. *Z*-acetamido[2-(phenyl sulphonyl)-ethenyl]malonate (Z-147a) (3.19g, 60%). M. p. 94-96^{0}C. δ_H (220 MHz; $CDCl_3$) 7.45 (6H, m., =C*H*Ph*H*), 5.54 (1H, d, J 12 Hz, C*H*=), 4.26 (4H, q, 7 Hz, C*H*$_2$ Et), 2.1 (3H, s, COC*H*$_3$), 1.25 (6H, t, J 7 Hz, C*H*$_3$ Et). δ_C (100 MHz, $CDCl_3$) 168.9 (C=O), 165.5 (C=O), 140.2 (C1 Ph), 133.8 (C4 Ph), 129.3 (Ph), 127 (Ph), 64.1 (*tert* C), 63.6 (CH_2), 22.1 (Ac CH_3), 13.8 (CH_3). ν_{max} ($CHCl_3$)/cm^{-1} 3418, 2254, 1748, 1678, 1271. *E*-acetamido[2-(phenylsulphonyl)-ethenyl]malonate (E-147a) (0.92g, 20%). M. p. 105-107^{0}C. δ_H (220 MHz; $CDCl_3$) 7.45 (6H, m., =C*H*Ph*H*), 7.1 (1H, br. s, N*H*), 6.4 (1H, d, J 16 Hz, C*H*=), 4.26 (2H, q, 7 Hz, C*H*$_2$ Et), 2.0 (3H, s, COC*H*$_3$), 1.25 (3H, t, J 7 Hz, C*H*$_3$ Et). *Z*-acetamido-[2-(phenylsulphonyl)-[3,4-$^2H_2$]-ethenyl]malonate (Z-147b) (3.19g, 60%). M. p. 94-96^{0}C. δ_H (220 MHz; $CDCl_3$) 7.45 (6H, m., N*H*, Ph*H*), 4.26 (4H, q, 7 Hz, C*H*$_2$ Et), 2.1 (3H, s, COC*H*$_3$), 1.25 (6H, t, J 7 Hz, C*H*$_3$ Et). *E*-acetamido[2-

(phenylsulphonyl)-[3,4-$^2H_2$]-ethenyl]malonate (E-147c) δ_H (220 MHz; $CDCl_3$) 7.45 (5H, m., Ph*H*), 7.05 (1H, br. s, N*H*), 4.26 (4H, q, 7 Hz, CH_2 Et), 2.05 (3H, s, $COCH_3$), 1.25 (6H, t, J 7 Hz, CH_3 Et). Agrees with literature data[92].

Preparation of diethyl acetamidoethenylmalonate (148)[62].

Aluminium foil (1.36g, 1.56mmol), cut into small pieces, was agitated in an aqueous mercury (II) chloride solution (0.5%, 20cm^3) for several minutes. The mercury chloride solution was decanted off and the shiny aluminium amalgam patted dry on filter paper. The amalgam was added to a solution of diethyl acetamido[2-(phenylsulphonyl)ethenyl]malonate (0.5g, 1.3mmol) in 1,4-dioxane (wet, 20cm^3) and stirred at room temperature for 2 days. The solution was filtered to remove a grey precipitate and concentrated under reduced pressure to give an oil which was distilled bulb to bulb under reduced pressure to give the title compound (148) (0.25g, 80%). B. p. 135°C 0.1 mm Hg. δ_H (220 MHz; $CDCl_3$) 7.0 (1H, br. s, N*H*), 6.58 (1H, dd, J 16 Hz, *CH*=), 5.35 (1H, d, J 12Hz, =CH*H*), 5.3 (1H, d, J 16Hz, =*CH*H), 4.26 (4H, q, 7 Hz, CH_2 Et), 2.1 (3H, s, $COCH_3$), 1.29 (6H, t, J 7 Hz, CH_3 Et). δ_C (100 MHz, $CDCl_3$) 168.5 (C=O), 166.9 (C=O), 116.2 (=C), 115.9 (=C), 66.9 (*tert* C), 62.8 (CH_2), 22.7 (Ac CH_3), 13.8 (CH_3). ν_{max} ($CHCl_3$)/cm^{-1} 3418, 1738, 1683, 1271. *E*-diethyl acetamido[2-(phenylsulphonyl)-[3,4-$^2H_2$]-ethenyl]malonate. δ_H (220 MHz; $CDCl_3$) 7.0 (1H, br. s, N*H*), 5.3 (1H, br. s, =*CH*D), 4.26 (4H, q, 7 Hz, CH_2 Et), 2.1 (3H, s, $COCH_3$), 1.29 (6H, t, J 7 Hz, CH_3 Et). *Z*-diethyl acetamido[2-(phenylsulphonyl)-[3,4-$^2H_2$]-ethenyl] malonate. δ_H (220 MHz; $CDCl_3$) 7.0 (1H, br. s, N*H*), 5.35 (1H, br. s, =CD*H*), 4.26 (4H, q, 7 Hz, CH_2 Et), 2.1 (3H, s, $COCH_3$), 1.29 (6H, t, J 7 Hz, CH_3 Et). Agrees with literature data[62].

Preparation of 2-amino-3-butenoic acid (71).

Diethyl acetamido[2-(phenylsulphonyl)ethenyl]malonate (0.41g, 1.69mmol) was refluxed in HCl (6 M, 15cm^3) for 1 hour. The solution was treated as for 2-amino-3-butenoic acid (in chapter 6.3) to give the title compound (71) (0.18g, 77%). δ_H (220 MHz; D_2O) 5.9 (1H, m, C*H*=CH_2), 5.45 (1H, d, J 15.7 Hz, CH=CH*H* trans), 5.43 (1H, d, J 12.5 Hz, CH=C*H*H cis), 4.3ppm (1H, d, J 9 Hz, C*H*-CH=CH_2). *Z*-[3,4-$^2H_2$]-2-amino-3-butenoic acid (220 MHz; D_2O) δ_H 5.5 (1H, br. s, CD=CD*H* cis), 4.3ppm (1H, br. s, C*H*-CD=). *E*-[3,4-$^2H_2$]-2-amino-3-butenoic acid (220 MHz; D_2O) δ_H 5.4 (1H, br. s, CD=C*H*D trans), 4.3ppm (1H, br. s, C*H*-CD=). Agrees with literature data[62].

Preparation of 2-(*N*-(*t*-butyloxycarbonyl)amino)-3-butenoic acid (143)[99].

As for (175) using di-*t*-butyl-dicarbonate and 2-amino-3-butenoic acid (0.101g, 1.0mmol) to give the title compound (0.193g, 96%). δ_H (220 MHz; $CDCl_3$) 7.1 (1H, m, N*H*), 6.0 (1H, m, C*H*=CH_2), 5.45 (1H, d, 19 Hz, CH=CH*H* trans), 5.30 (1H, d, J 10 Hz, CH=C*H*H cis), 4.87 (1H, m, C*H*), 1.41 (9H, s, *t*-Bu). *E*-[3,4-$^2H_2$]-2-(*N*-(*t*-butyloxycarbonyl)amino)-3-butenoic acid δ_H (250 MHz; $CDCl_3$) 7.1 (1H, m, N*H*), 5.37 (0.72H, unres. t, CD=C*H*D trans), 5.30 (0.28H, br. s, CD=CD*H* cis), 4.87 (1H, m, C*H*), 1.41 (9H, s, *t*-Bu). *Z*-[3,4-$^2H_2$]-2-*N*-(*t*-butyloxycarbonyl)amino)-3-butenoic acid δ_H (220 MHz; $CDCl_3$) 7.1 (1H, m, N*H*), 5.37 (0.6H, br. s, CD=C*H*D trans), 5.30 (0.3H, br. s, CD=CD*H* cis), 4.87 (1H, m, C*H*), 1.41 (9H, s, *t*-Bu).

6.2 : EXPERIMENTAL DETAILS FOR CHAPTER FOUR.

Preparation of methyl-3-butenylimidate hydrochloride (153)[107].

Gaseous hydrogen chloride (dry) was bubbled through the solution of 3-propenenitrile (freshly distilled, 3.5g, 52mmol) and methanol (superdry, 3.5cm^3) and was stirred under a nitrogen atmosphere at 0^{o}C for one hour. The vessel was tightly sealed and allowed to stir overnight at 4^{o}C. Cold diethyl ether (dry, 200cm^3) was added causing the product to precipitate from solution which was removed by filtration. The cake was washed with further ether (2 x 50cm^3). The white hygroscopic solid was dried under high vacuum to give the title compound (153) (6.86g, 97%). δ_H (220 MHz; D_2O) 5.9 (1H, m, C*H*=CH_2), 5.25 (1H, br. d, J 16 Hz, CH=C*H*H trans), 5.25 (1H, br. d, J 12 Hz, CH=CH*H* cis), 3.7 (3H, s, OCH_3), 3.17ppm (2H, d, J 12 Hz, CH_2). ν_{max} (nujol mull)/cm^{-1} 1669 (C=N). m/z (CI) 136 ((M+1)$^+$, 10.1%), 122 (5.4), 100 ((M-HCl)$^+$, 100), 86 ((M-HCl-CH_3)$^+$, 47.1), 68 (2.0). m/z (EI) 235((2M-HCl)$^+$, 2.5%), 202 ((2M+2-2HCl)$^+$, 7.1), 185 ((2M+1-HCl-CH_3)$^+$, 16.9), 136 ((M+1)$^+$, 28.7), 110 (39.7), 100 ((M-HCl)$^+$, 100), 93 ((2M+1-2HCl-CH_3)$^+$, 63.7), 86 ((M+1-HCl-CH_3)$^+$, 1.2).

Preparation of methyl *N*-chloro 3-butenylimidate (154).

Imidate hydrochloride (153) (3.0g, 22mmol) was added directly to a vigorously stirred solution of sodium hypochlorite solution (fresh, 12-14% available chlorine, 150cm^3) at 0^{o}C under a nitrogen atmosphere. After stirring at this temperature for one hour the solution was extracted with petroleum ether (40-60^o fraction, 2 x 50cm^3). The organic phase was dried with magnesium sulphate and evaporated under reduced pressure to give the title compound (154) as a colourless oil which was

used without further purification. (2.98g, 100%). δ_H (220 MHz; $CDCl_3$) 5.9 (1H, m, C*H*=CH_2), 5.28 (1H, d, J 17 Hz, CH=C*H*H trans), 5.25 (2H, d, J 11 Hz, CH=CH*H* cis), 3.85 (3H, s, OCH_3), 3.4ppm (2H, d, J 12 Hz, CH_2). m/z (EI) 134 ($(M+1)^+$, 6.9%), 98 ($(M-HCl)^+$, 20.6), 92 (17), 83 (0.7), 69 (100).

Preparation of (*R,S*)-2-aminobut-3-enoic acid (71).

A solution of methyl *N*-chloro-3-butenylimidate (154) (0.882g, 6.6 mmol) and sodium hydroxide (3 equivalents, 1.303g, 33 mmol) in water ($150cm^3$) was stirred overnight at 2°C. The solution was passed over a column of Dowex 50W-8X (H) and washed with water ($250cm^3$). The product was eluted with 5% aqueous pyridine solution to give the amino acid (0.3542g, 53%) pure by ^1H nmr which was recrystallised from water / ethanol to give the title product (71) (0.2186g, 33%). M. p. 175°C(d). δ_H (220 MHz; D_2O) δ_H 5.9 (1H, m, C*H*=CH_2), 5.45 (1H, d, J 15.7 Hz, CH=C*H*H trans), 5.43 (1H, d, J 12.5 Hz, CH=CH*H* cis), 4.3ppm (1H, d, J 9 Hz, C*H*-CH=CH_2). δ_C (100 MHz, D_2O) 173.7 (C1), 131.0 (C3), 122.1 (C4), 57.8 (C2). m/z (CI) 102, ($(M+1)^+$, 20.1%), 58 ($(M+1-CO_2)^+$, 1.1), 56 (9.1) (Found $M+1^+$: 102.0555. $C_4H_8NO_2$ requires 102.0555). Agrees with literature data[106].

Preparation of (*R,S*)-2-(*N*-*t*-butyloxycarbonyl-amino)but-3-enoic acid (143).

A solution of methyl *N*-chloro-3-butenylimidate (154) (1.1g, 8.9mmol) and sodium hydroxide (3 eq., 1.079g, 27mmol) in water ($50cm^3$) was stirred overnight at 2°C. 1,4-dioxane ($50cm^3$) was added followed by di-*t*-butyl dicarbonate (1.2 eq., 2.136g, 9.79mmol) and allowed to stir for 8 hours at 2°C. The 1,4-dioxane was removed *in vacuo* and the resultant aqueous solution washed with ethyl acetate ($10cm^3$). The aqueous phase

was layered with ethyl acetate ($25cm^3$) and acidified to pH 2 with cold 1 M potassium bisulphate solution The aqueous layer was washed with further ethyl acetate (2 x $25cm^3$). The combined organic phases were washed with water ($10cm^3$) and saturated brine ($10cm^3$), dried with magnesium sulphate and concentrated under reduced pressure to give the product as a straw coloured oil (143) (1.105g, 67%). δ_H (220 MHz; $CDCl_3$) 7.1 (1H, m, *NH*), 6.0 (1H, m, *CH*=CH_2), 5.45 (1H, d, 19 Hz, CH=*CH*H trans), 5.30 (1H, d, J 10 Hz, m, CH=*CH*H cis), 4.87 (1H, m, *CH*), 1.41 (9H, s, *t*-Bu). δ_C (100 MHz, $CDCl_3$) 174.5, 173.6 (C1), 156.7, 155.2 (C=O Boc), 132.6, 132.2 (C3), 117.5 (C4), 81.6, 80.3 (C-Me_3 Boc), 57.0, 55.6 (C2), 28.1 (CH_3 Boc). m/z (CI) 202 ($(M+1)^+$, 2.4%), 163 (20), 146 ($(M-C_4H_8)^+$, 6.5), 102 ($(2M-CO_2C_4H_8)^+$, 4.9), 86 (0.6). (Found $M+1^+$: 202.1067. $C_9H_{16}NO_4$ requires 202.1079). m/z (EI) 202 ($(M+1)^+$, 20.1%), 58 ($(M+1-CO_2)^+$, 1.1), 56 (9.1). ν_{max} ($CHCl_3$)/cm^{-1} 1720, 1510, 1465.

Preparation of ethyl-(*S*)-2-(*N*-*t*-butyloxycarbonyl)aminobut-3-enoate (144)[96].

A mixture of citrate/phosphate buffer solution (1M, pH 4.2, 15ml), papain (ex-Sigma, type 2 crude, 0.3g), L-cysteine (45mg) and ethylenediamine tetraacetic acid tetra sodium salt (1 M, 75μL) were allowed to stir for 10 minutes. *R,S*-acid (143) (0.5g, 2.5mmol) in dichloromethane ($3cm^3$) and ethanol ($2cm^3$) were added and the solution was stirred at 37°C for 2 days. The reaction mix was filtered through a bed of Celite and the cake washed with ethyl acetate ($15cm^3$) and water ($15cm^3$). The organic layer was separated off and the aqueous phase extracted with ethyl acetate ($25cm^3$) and acidified with 1 M potassium bisulphate solution until blue to Congo red. The combined organic phase was washed with water ($10cm^3$) and saturated brine ($10cm^3$). The crude (*R*)-acid was removed by

washing the organic layer with 1 M sodium bicarbonate solution ($2 \times 5 cm^3$) and after acidification was subjected to a further incubation with papain as above. The combined organic phases of both runs were washed with saturated brine ($10 cm^3$), and dried with magnesium sulphate and concentrated under reduced pressure to give an residue which was flash chromatographed with ethyl acetate / petroleum ether 1 : 9 solvent system to give an oil (144) (0.2g, 80%). δ_H (400 MHz; $CDCl_3$) 5.83 (1H, ddd, J 17.1, 10.4, 5.4 Hz, *CH*=CH_2), 5.35 (1H, dd, 17.1, 1.8 Hz, CH=C*H*H trans), 5.30 (1H, dd, J 10.3, 1.7 Hz, CH=CH*H* cis), 4.8 (1H, m, C*H*), 4.17 (2H, q, J 7.1 Hz, CH_2CH_3), 1.41 (9H, s, *t*-Bu), 1.23 (3H, t, J 7.1 Hz, CH_2CH_3). δ_C (100 MHz, $CDCl_3$) 170.6 (C1), 154.7, 155.2 (C=O Boc), 132.7 (C3), 117.0 (C4), 79.9 (C-Me_3 Boc), 61.6 (C2), 55.7 (CH_2 Et), 28.1 (CH_3 Boc), 14.0 (CH_3 Et). m/z (CI) 230 ($(M+1)^+$, 0.8%), 174 ($(M+1-C_4H_8)^+$, 18.2), 156 (13.8), 130 ($(M+1-C_4H_8-CO_2)^+$, 9.2), 100 ($(M+1-C_4H_8-CO_2-C_2H_5)^+$, 23.1), 56 (2.8%), (Found $M+1^+$: 230.1394. $C_{11}H_{20}NO_4$ requires 230.1392). ν_{max} ($CHCl_3$)/cm^{-1} 1770, 1712, 1495 cm^{-1}.

The optical purities of the resolved amino acid derivatives was examined by the optical rotation of the free amino acid prepared by the usual method of acid hydrolysis and ion exchange with Dowex 50W-8X (H^+).

(*S*)-2-amino-3-butenoic acid : $[\alpha]_D$ = +94.7° (H_2O, 25.8°C, c=0.25).

(*R*)-2-amino-3-butenoic acid : $[\alpha]_D$ = +96.3° (H_2O, 22.9°C, c=0.46).

Preparation of methyl *N*-chloro-3-pentenylimidate (156).

As for (153) using freshly distilled 3-pentenenitrile (155) (5.0g, 61.7mmol) to give the title compound (156) (8.03g, 87%). δ_H (400 MHz; $CDCl_3$) 5.75 (2H, m, C*H*=C*H*), 3.77 (3H, s, OCH_3), 3.04 (2H, d, J 7 Hz, CH_2), 1.75 (3H, d, J 5 Hz, CH_3). m/z (EI) 263 ($(2M+1-HCl)^+$, 1.8%), 149 ($(M)^+$, 0.6), 114 ($(M-Cl)^+$, 100), 99 (7.5), 93 (15), 75 (7.4), 55 (13.9) (Found $M+1^+$:

150.0658. $C_6H_{13}ClNO$ requires 150.0677). ν_{max} ($CHCl_3$)/cm^{-1} 3176, 1660, 1475.

Preparation of methyl *N*-chloro-3-pentenylimidate (157).

As for (154) using fresh methyl-3-pentenylimidate hydrochloride (156) (2.0g, 13.4mmol) to give the title compound (157) (1.91g, 97%). (400 MHz; $CDCl_3$) δ_H (400 MHz; $CDCl_3$) 5.6 (1H, m, C*H*=CH), 5.45 (1H, m, CH=C*H*), 3.76 (3H, s, OCH_3), 3.25 (2H, ddd, J 6.7, 1.3, 1.3 Hz, CH_2), 1.67 (3H, q, ddt, J 6.4, 1.3, 1.3 Hz, CH_3). δ_C (100 MHz, $CDCl_3$) 174.6 (C-1), 129.6 (C-3), 121.7 (C-4), 54.7 (C-2), 35.2 (OCH_3), 17.8 (C5-H). m/z (EI) 147 ($(M)^+$, 3.24%), 132 ($(M-CH_3)^+$, 2.99), 112 ($(M-HCl)^+$, 4.85), 97 (1.27) (Found M^+ : 147.0441. $C_6H_{10}ClNO$ requires 147.045). m/z (CI) 148($(M+1)^+$, 21.63%), 132 ($(M-CH_3)^+$, 1.06), 112-($(M-HCl)^+$, 2.43). ν_{max} ($CHCl_3$)/cm^{-1} 2950, 1605, 1443.

Preparation of 2-amino-3-pentenoic acid (158).

As for (85) using methyl *N*-chloro-3-pentenylimidate (157) (0.8g, 5.4mmol) to give the title compound (158) (0.084g, 14%). M. p. 178°C (d). δ_H (250 MHz; D_2O) 5.9 (1H, ddq, J 6.5, 15.3, 0.8 Hz, C*H*=CH), 5.35 (1H, ddq, 8.5, 1.7, 15.3 Hz, CH=C*H*), 4.1 (1H, d, J 8.5 Hz, CH_2), 1.6 (3H, dd, J 6.5, 1.7 Hz, CH_3). δ_C (63 MHz, $CDCl_3$) 174.5 (C1), 135.9 (C3), 123.4 (C4), 57.9 (C2), 18.0 (C5). m/z (EI) 116 ($(M+1)^+$, 10.6%), 70 ($(M-CO_2)^+$, 2.4), 35 (10.5) (Found $M+1^+$: 116.0713. $C_5H_{10}NO_2$ requires 116.071).

Preparation of 2-(*N*-*t*-butyloxycarbonyl)amino-3-pentenoic acid (159).

As for (143) using methyl *N*-chloro-3-pentenylimidate (157) (0.6g, 4.5mmol) to give the title compound (159) (0.35g, 29%). δ_H (250 MHz; $CDCl_3$) 5.8 (1H, dqd, 15.26, 6.32, 1.11 Hz, *CH*CH_3), 5.51 (1H, m, N*H*), 5.45, 5.30 (1H, unres. dd, J 15, 5 Hz, *CH*=CHCH$_3$), 4.75, 4.54 (1H, 2xm, *CH*), 1.67 (3H, d, 6.7, *CH*$_3$), 1.39 (9H, s, *t*-Bu). δ_C (100 MHz, $CDCl_3$) 177.5, 175.2 (C1), 156.6, 155.1 (C=O Boc), 130.0, 129.7 (C3), 125.0 (C4), 81.5, 80.1 (C-Me$_3$ Boc), 56.6, 55.1 (C-2), 28.1 (CH_3 Boc), 17.6 (C-5).

Preparation of ethyl (*S*)-2-(*N*-*t*-butyloxycarbonyl)amino)-3-pentenoate (160).

As for (144) using (*R*,*S*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-pentenoate (0.3g, 1.4mmol) to give the (*R*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-pentenoate (0.84g, 56%) and the title compound ethyl (*S*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-pentenoate (160) (0.13g, 76%). δ_H (250 MHz; $CDCl_3$) 5.71 (1H, dqd, J 14.3, 6.56, 1.4 Hz, CH=*CH*CH$_3$), 5.35 (1H, unres. dd, 15.5, 4.8 Hz, *CH*=CHCH$_3$), 5.1 (1H, m, N*H*), 4.67 (1H, m, *CH*), 4.17 (2H, q, J 7.0 Hz, *CH*$_2$ Et), 1.65 (3H, d, J 5.1Hz, *CH*$_3$), 1.41 (9H, s, *t*-Bu), 1.23 (3H, t, J 7.0 Hz, *CH*$_3$ Et). δ_C (63 MHz, solvent $CDCl_3$) 129.2 (C3), 125.5 (C4), 79.9 (C-Me$_3$ Boc), 61.4 (C2), 55.7 (CH_2 Et), 28.2 (CH_3 Boc), 17.6 (C5), 14.0 (CH_3 Et). m/z (CI) 244 ((M+1)$^+$, 0.8%), 205 (30.6), 188 ((M-C_4H_8)$^+$, 86.8), 170 ((M-C_4H_8-CO_2)$^+$, 48.3), 144 ((M-C_4H_8-CO_2-C_2H_5)$^+$, 87.7), 127 (20.3), 114 ((M-C_4H_8-CO_2-C_2H_5)$^+$, 29), 99 (6.3), 70 ((M-C_4H_8-CO_2-C_2H_5-CO_2)$^+$, 25.5). m/z (EI) 170 ((M-C_4H_8-CO_2)$^+$, 38.4%), 134 (15.1), 127 (27), 114 ((M-C_4H_8-CO_2-C_2H_5)$^+$, 93.6), 83 (100), 70 ((M-C_4H_8-CO_2-C_2H_5-CO_2)$^+$, 25.5). ν_{max} ($CHCl_3$)/cm^{-1} 1760, 1708, 1493.

The optical purities of the resolved amino acid derivatives were examined by the optical rotation of the free amino acid prepared by the usual method of acid hydrolysis and ion exchange with Dowex 50W-8X (H^+).

(*S*)-2-amino-3-pentenoic acid : $[\alpha]_D$ = +111.9° (H_2O, 25.6°C, c=0.2).

(*R*)-2-amino-3-pentenoic acid : $[\alpha]_D$ = +114.2° (H_2O, 29.5°C, c=0.2).

Preparation of 2-methyl-2-butene-1-ol (162).

Methyl tiglate (161) (5.8g, 51mmol) was added to a stirred ethereal (60cm^3) solution of lithium aluminium hydride (1.87g, 51mmol) at 0°C and stirred for two hours. The excess lithium aluminium hydride was destroyed by the careful addition of ethyl acetate (10cm^3) and the suspension was filtered and washed with saturated brine to give the title compound (162) which was used without further purification. (3.65g, 85%). δ_H (220 MHz; $CDCl_3$) 5.54 (1H, q, J 7 Hz, C=C*H*), 4.02 (2H, s, C*H*$_2$), 1.69 (3H, s, C-C*H*$_3$), 1.69 (3H, d, J 7 Hz, CH-C*H*$_3$).

Preparation of 1-bromo-2-methyl-2-butene (163).

Phosphorous tribromide (0.33eq., 2.97cm^3, 28mmol) was added carefully to a solution of 2-methyl-2-butene-1-ol (162) (7.39g, 86mmol) in diethyl ether (200cm^3) at 0°C the solution was allowed to stir overnight at room temperature. The mixture was washed with water (30cm^3) and saturated brine (30cm^3), dried over magnesium sulphate and reduced *in vacuo*. The residue was distilled to give the title compound (163) (8.5g, 67%). B. p. 74°C 18 mm Hg. δ_H (220 MHz; $CDCl_3$) 5.74 (1H, q, J 8 Hz, C*H*-CH_3), 4.01 (2H, s, C*H*$_2$), 1.78 (3H, s, C*H*$_3$), 1.64 (3H, d, J 8 Hz, CH-C*H*$_3$). Agrees with literature data[150].

Preparation of 3-methyl-3-pentenenitrile (164).

1-Bromo-2-methyl-2-butene (163) (5.3g, 36mmol) was added to a solution of sodium cyanide (1.1eq., 2.1g, 43mmol) in DMSO (30cm^3) at 50°C, and the solution allowed to stir until it reached room temperature. The mixture was diluted with water (250cm^3) and extracted with petroleum ether (4 x 25cm^3). The organic phase was washed with water (30cm^3) and saturated brine (30cm^3), dried over magnesium sulphate and concentrated under reduced pressure. The residue was distilled to give the title compound (164) (2.5g, 73%). B. p. 54°C 18 mm Hg. δ_H (220 MHz; $CDCl_3$) 5.6 (1H, q, J 7 Hz, C*H*-CH_3), 3.04 (2H, s, CH_2), 1.73 (3H, s, CH_3), 1.64 (3H, d, J 7 Hz, CH-CH_3). Agrees with literature data[150].

Preparation of methyl 3-methyl-3-pentenylimidate hydrochloride (165).

As for (153) using 3-methyl-3-pentenenitrile (164) (1.63g, 17mmol) to give the title compound (165) (2.75g, 100%). δ_H (220 MHz; D_2O) 5.15 (1H, q, J 2.8 Hz, C*H*-CH_3), 4.21 (3H, s, OCH_3), 3.33 (2H, s, CH_2), 1.7 (3H, s, CH_3), 1.68 (3H, d, J 2.8Hz, CH-CH_3). m/z (EI FAB) 291 ((2M-HCl)$^+$, 0.9%), 256 ((2M-2Cl), 1.1), 220 (2.6), 185 (8.9), 164 ((M+1)$^+$, 26.3), 149 ((M-CH_3)$^+$, 0.9), 128 ((M-Cl)$^+$, 100), 110 (3.1). ν_{max} (nujol mull)/cm^{-1} 1656, 1465, 1448 cm^{-1}.

Preparation of methyl *N*-chloro-3-methyl-3-pentenylimidate (166).

As for (154) using methyl 3-methyl-3-pentenylimidate hydrochloride (165) (1.0g, 6.1mmol) to give the title compound (166) (1.0g, 100%). δ_H (400 MHz; $CDCl_3$) 5.28 (1H, qq, J 6.7, 1.3 Hz, C*H*-CH_3), 3.76, 3.74 (1H, s, OCH_3), 3.22 (2H, br. s, CH_2), 1.6 (3H, br. s, CH_3), 1.58 (3H, d, J 6.7 Hz, CH-CH_3). δ_C (100 MHz, $CDCl_3$) 174 (C1), 128.5, 128.1 (C3), 122.9, 128.1 (C4), 56.8, 54.4

(C2), 41.1, 40.4 (OCH_3), 15.7 (C5), 13.3 (C6). m/z (CI) 178 ($(M+NH_3)^+$, 1.3%), 162 ($(M+1)^+$, 56.6), 146 ($(M-CH_3)^+$, 1.1), 126 ($(M-Cl)^+$, 12.4), 111 ($(M-CH_3-Cl)^+$, 0.8), 85 ($(M-CH_3-Cl-CN)^+$, 0.8). m/z (EI) 162 ($(M+1)^+$, 39.5%), 146 ($(M-CH_3)^+$, 54.2), 126 ($(M-Cl)^+$, 100), 111 ($(M-CH_3-Cl)^+$, 32.2), 94 ($(M-Cl-OCH_3)$, 54.3), 85 ($(M-CH_3-Cl-CN)^+$, 54.3). ν_{max} (nujol mull)/cm^{-1} 1608, 1445.

Preparation of 2-amino-3-methyl-3-pentenoic acid (167).

As for (85) using methyl *N*-chloro-3-methyl-3-pentenylimidate (166) (0.46g, 2.9mmol) to give the title compound (167) (0.22g, 100%). δ_H (400 MHz; D_2O) 5.71 (1H, qq, J 6.7, 1.3 Hz, C*H*-CH_3), 4.11 (1H, s, C*H*), 1.6 (3H, dq, J 1.0, 6.7Hz, CH-C*H*$_3$), 1.56 (3H, dq, J 1.2, 1.0Hz, C*H*$_3$). δ_C (100 MHz, $CDCl_3$) 174.1 (C1), 130.7 (C4), 128.8 (C3), 62.9 (C2), 13.7 (C5), 11.8 (C6). m/z (EI FAB) 259 ($(2M+1)^+$, 10.6%), 222 (11.0), 185 (7.2), 130 ($(M+1)^+$, 100), 113 ($(M-OH)^+$, 14.4), 93 ($(M-OH-CO)^+$, 10).

Preparation of 2-(*N*-*t*-butyloxycarbonyl)amino-3-methyl-3-pentenoic acid (168)[107].

Methyl *N*-chloro-3-methyl-3-pentenylimidate (167) (0.79g, 4.8mmol) in petroleum ether 40-60° ($3cm^3$) was added to sodium (3eq., 0.34g, 14.6mmol) in methanol ($6cm^3$) and stirred at room temperature for 24 hours. The solution was diluted with water ($20cm^3$) and 1,4-dioxane ($20cm^3$) and di-*t*-butyl-dicarbonate (1.2 eq., 1.27g, 5.8mmol) and worked up as usual to give the title compound (168) (2.75g, 100%). δ_H (250 MHz; $CDCl_3$) 5.63 (1H, m, C*H*-CH_3), 5.26 (1H, m, N*H*), 4.7, 4.51 (1H, m, C*H*), 1.64 (3H, s, C*H*$_3$), 1.63 (3H,d, J 6 Hz, CH-C*H*$_3$), 1.42 (9H, s, *t*-Bu). δ_C (63 MHz, $CDCl_3$) 175.2, 174.0 (C1), 155.4 (C=O Boc), 130.6 (C4), 125.2, 125.1 (C3), 80.2 (C-Me_3 Boc), 61.69, 60.31 (C2), 28.3 (CH_3 Boc), 13.5 (C5), 12.9 (C6). m/z (CI)

230 ((M+1)$^+$, 5.6%), 191 (48.2), 174 ((M-C_4H_8)$^+$, 51.3), 146 ((M-C_4H_8-CO)$^+$, 5.2), 130 ((M-C_4H_8-CO_2)$^+$, 17.4), 112 (8.4), 84 ((M-$CO_2C_4H_9$-CO_2)$^+$, 43.8), 79 (5.9). m/z (EI) 173 ((M-C_4H_8)$^+$, 5.7%), 128 ((M-$CO_2C_4H_9$,)$^+$, 53), 112 (6.6), 84 ((M-$CO_2C_4H_9$-CO_2)$^+$, 35.8). ν_{max} ($CHCl_3$)/cm^{-1} 1715s, 1655s, 1456.

Preparation of ethyl (*S*)-2-(*N*-*t*-butyloxycarbonyl)amino-3-methyl-3-pentenoate (169).

As for (144) with 2-(*N*-*t*-butyloxycarbonyl)amino-3-methyl-3-pentenoic acid (168) (0.23g, 1.0mmol) to give the title compound (169) as a straw coloured oil (69mg, 53%). δ_H (400 MHz; $CDCl_3$) 5.57 (1H, br. q, J 5.9 Hz, C*H*-CH_3), 5.27 (1H, br s, N*H*), 4.65 (1H, br. d, J 7.3 Hz, C*H*), 4.17 (2H, q, J 7.1 Hz, CH_2 Et), 1.62 (3H, d, J 6.0 Hz, CH-CH_3), 1.6 (3H, s, CH_3), 1.42 (9H, s, *t*-Bu), 1.23 (3H, t, J 7.1 Hz, CH_3 Et). δ_C (100 MHz, $CDCl_3$) 171.3 (C1), 154.8 (C=O Boc), 131.0 (C3), 124.3 (C4), 79.7 (C-Me_3 Boc), 61.3 (C2), 60.4 (CH_2 Et), 30.8 (CH_3 Boc), 14.0 (CH_3 Et), 13.4 (C5), 12.7 (C6). m/z (CI) 258 ((M+1)$^+$, 11.6), 219 (31.4), 202 ((M-C_4H_8)$^+$, 52.8), 158 ((M-C_4H_8-CO_2)$^+$, 85.3), 157 ((M-$CO_2C_4H_8$)$^+$, 3.6), 110 (1.1), 84 ((M-C_4H_8-CO_2-$C_2H_5.CO_2$)$^+$, 39.7). ν_{max} ($CHCl_3$)/cm^{-1} 2928, 1712, 1496.

The optical purity of the resolved amino acid derivative was examined by the optical rotation of the free amino acid prepared by the usual method of acid hydrolysis and ion exchange with Dowex 50W-8X (H^+).

(*S*)-2-aminopentenoic acid : $|\alpha|_D$ = +65.4° (H_2O, 24.1°C, c=0.26).

Preparation of 3-methyl-propenenitrile (171)[111].

Copper (I) cyanide (8.96g, 0.1mol) was added slowly to a solution of 3-chloro-2-methyl-1-propene (9.1g, 0.1mol) in nitrobenzene (freshly distilled, 40cm^3) at 125°C for two hours. The title compound (171) was

purified by fractional distillation (6.26g, 77%). B. p. 84-86°C 18 mm Hg. δ_H (220 MHz; $CDCl_3$) 5.14 (1H, br. s, C=C*H*H), 5.05 (1H, br. s, C=CH*H*), 3.11 (2H, s, CH_2), 1.87 (3H, s, CH_3). Agrees with literature data[111].

Preparation of methyl 3-methyl-3-propenylimidate hydrochloride (172).

As for (153) using 3-methyl-3-propenenitrile (171) (2.0g, 25mmol) to give the title compound (172) (3.6g, 100%). δ_H (220 MHz; D_2O) 5.18 (1H, br s, C=C*H*H), 5.03 (1H, br s, C=CH*H*), 4.2 (3H, s, OCH_3), 3.42 (2H, s, CH_2), 1.8 (3H, s, CH_3). m/z (EI FAB) 263 ((2M-HCl), 1.7%), 227 ((2M-2HCl)$^+$, 1.48), 136 (1.39), 114 ((M-HCl)$^+$, 100), 89 (1.22), 73 (4.37). ν_{max} (nujol mull)/cm^{-1} 1806, 1660, 1466.

Preparation of methyl *N*-chloro-3-methyl-3-propenylimidate (173).

As for (154) using methyl *N*-chloro-3-methyl-propenylimidate hydrochloride (172) (1.0, 6.8mmol) to give the title compound (173) (1.0g, 87%). δ_H (220 MHz; $CDCl_3$) 5.02, 4.93 (1H, br s, C=C*H*H), 4.87, 4.8 (1H, br s, C=CH*H*), 3.94, 3.8 (3H, s, OCH_3), 3.24, 3.0 (2H, s, CH_2), 1.79,1.76 (3H, s, CH_3). δ_C (100 MHz, $CDCl_3$) 174.1 (C1), 138.3, 137.8 (C4), 114.1, 113.7 (C3), 57.0, 54.7 (C2), 39.8, 39.1 (C5), 22.4, 22.0 (OCH_3). m/z (CI) 148 ((M+1)$^+$, 17.2%), 112 ((M-HCl)$^+$, 65.0), 97 ((M-HCl-CH_3)$^+$, 3.7), 84 (9.3), 72 (12.5). m/z (EI) 148 ((M+1)$^+$, 17.2%), 112 ((M-HCl)$^+$, 65.0), 92 (12.2), 81 ((M-HCl-OCH_3)$^+$, 16.0), 77 (12.5%). ν_{max} ($CHCl_3$)/cm^{-1} 1608, 1446, 1379.

Preparation of 2-amino-3-methyl-3-propenoic acid (174)[107].

Methyl *N*-chloro-3-methyl-3-propenylmidate (173) (0.4g, 2.7mmol) was dissolved in petroleum ether 40-60° (5cm^3) and added to a solution of sodium (3 eq., 1.86g, 81mmol) in methanol (6cm^3). The solution was stirred overnight at room temperature. Water (10cm^3) was added to the mixture and the solution acidified with M HCl. The organic layer was removed and the solution passed down a Dowex 50W 8X (H^+) ion exchange column and the product eluted with 4% aqueous pyridine to give the title compound (174) which was recrystallised from ethanol / water (0.24g, 76%). M. p. 186°C (d). δ_H (400 MHz; D_2O) 5.51 (1H, dq, J 1.2 Hz, C=C*H*H), 5.25 (1H, d, J 1 Hz, C=CH*H*), 4.19 (1H, s, C=C*H*), 1.72 (1H, dd, J 2,1Hz, C*H*$_3$). δ_C (100 MHz, solvent D_2O) 173.4 (C1), 148.6 (C3), 129.5 (C4), 60.9 (C2), 55.7 (C5). m/z (EI FAB) 116 ((M+1)$^+$, 67%), 93 (100), 75 (47.6), 70 (39.2), 58 (40.7). Agrees with literature data[107].

Preparation of 2-(*N*-*t*-butyloxycarbonyl)amino-3-methyl-3-propenoic acid (175).

As for (143) using di-*t*-butyl-dicarbonate and 2-amino-3-propenoic acid (0.282g, 2.5mmol) to give the title compound (175) (0.53g, 100%). δ_H (220 MHz; $CDCl_3$) 5.41 (1H, m, N*H*), 5.1 (1H, br s, C=C*H*H), 5.02 (1H, br s, C=CH*H*), 4.74, 4.58 (1H, m, C*H*), 1.7 (3H, s, C*H*$_3$), 1.31 (9H, s, *t*-Bu). δ_C (100 MHz, $CDCl_3$) 174.4, 173.3 (C1), 155.0 (C=O Boc), 140.0 (C4), 114.9 (C3), 80.2 (C-Me$_3$ Boc), 60.1, 58.8 (C2), 28.1 (CH_3 Boc), 19.2 (C5). m/z (CI) 216 ((M+1)$^+$, 1.4%), 177 (9.8), 160 ((M-C_4H_8)$^+$, 4.7), 116 ((M-C_4H_8-CO_2)$^+$, 6.7), 97 (1.3), 70 ((M-$CO_2C_4H_9$-CO_2)$^+$, 39.1). m/z (EI) 159 ((M-C_4H_8)$^+$, 21.9%), 114 (80.1), 96 (12.4), 83 (23.4), 83 (100), 70 ((M-$CO_2C_4H_9$-CO_2)$^+$, 72.3). ν_{max} ($CHCl_3$)/cm^{-1} 1718, 1455.

Preparation of 1-bromo-3-methyl-2-butene (177).

As for (163) using 3-methyl-2-butene-1-ol (176) (4.3g, 50mmol) to give the title compound (177) (5.68g, 76%). B. p. 66°C 18 mm Hg. δ_H (220 MHz; $CDCl_3$) 5.55 (1H, t, J 8 Hz, C*H*=C), 4.04 (2H, d, J 8 Hz, C*H*$_2$), 1.79 (3H, s, C=C*H*$_3$), 1.74 (3H, s, C=C*H*$_3$).

Preparation of 4-methyl-3-pentenenitrile (178).

As for (164) using 1-bromo-3-methyl-2-butene (177) (3.78g, 25mmol) to give the title compound (178) (2.0g, 83%). B. p. 130°C 18 mm Hg. δ_H (220 MHz; $CDCl_3$) 5.21 (1H, t, J 7 Hz, C*H*=C), 3.08 (2H, d, J 7 Hz, C*H*$_2$), 1.79 (3H, s, C=C*H*$_3$), 1.64 (3H, s, C=C*H*$_3$). Agrees with literature data[151].

Preparation of 5,5'-dimethyl-2-methoxy-1-pyrrolinium hydrochloride (180).

As for (153) with 4-methyl-3-pentenenitrile (178) (3.0g, 31.5mmol) to give the title compound (180) as a white solid (5.1g, 100%). δ_H (400 MHz; $CDCl_3$) 3.64 (3H, s, OC*H*$_3$), 2.42 (2H, q, AA'BB', C*H*$_2$), 1.94 (2H, q, AA'BB', C*H*$_2$), 1.52 (6H, s, C(C*H*$_3$)$_2$). δ_C (100 MHz, $CDCl_3$) 172.2 (C1), 69.2 (C4), 52.6 (C3), 41.8 (C2), 32.1 (2xCH$_3$), 31.5 (OCH$_3$). m/z (CI) 164 ((M+1)$^+$, 17.1%), 150 (0.8), 128 ((M-HCl)$^+$, 9.8), 114 ((M-HCl-CH$_3$)$^+$, 1.6), 96 ((M-HCl-OCH$_3$)$^+$, 1.3), 73 (2.3%). m/z (EI) 128 ((M-HCl)$^+$, 24%), 112 (5), 96 ((M-HCl-OCH$_3$)$^+$, 14), 86 (4), 77 (3.5). ν_{max} (choroform)/cm^{-1} 1643, 1435, 1090 cm^{-1}.

Preparation of 2-(*N*-*t*-butyloxycarbonyl)amino-3,4-epoxy-butanoate (181)[113].

A solution of *m*-chloroperbenzoic acid (purified, 1.6 eq., 0.23g, 1.3mmol) was added to ethyl (*S*)-2-(*N*-*t*-butyloxycarbonyl)-amino-3-butenoate (0.19g, 0.82mmol) in carbon tetrachloride (5cm^3) at 0°C under nitrogen and stirred for two hours. The mixture was concentrated under reduced pressure and purified by flash chromatography ethyl acetate / petroleum ether 1 : 10 to give the title compound as an oil (181) (0.123g, 60%). δ_H (250 MHz; $CDCl_3$) 5.0 (1H, br d, J 8.5 Hz, NH), 4.60 (1H, dd, J 8.9, 1.7 Hz, O_2CCH), 4.23 (2H, q, J 7.1 Hz, CH_2 Et), 3.42 (1H, m, $CHCH$), 2.75 (1H, dd, J 4.5, 4.3 Hz, CHH), 2.64 (1H, dd, J 4.5, 2.5 Hz, CHH), 1.42, 1.41 (9H, s, *t*-Bu), 1.28 (3H, t, J 7.1Hz, CH_3 Et). δ_C (100 MHz, $CDCl_3$) 169.8 (C1), 155.3 (C=O), 80.1 (C-Me_3 Boc), 61.7 (C2), 52.7 (CH_2 Et), 51.1 (C3), 43.6 (C4), 28.2 (CH_3 Boc), 13.9 (CH_3 Et). m/z (CI) 246 ((M+1)$^+$, 17.7%), 190 ((M-C_4H_8)$^+$, 99.1), 172 ((M-$CO_2C_2H_5$)$^+$, 22.2), 146 ((M-C_4H_8-CO_2)$^+$, 8.9), 128 (3.9), 116 ((M-C_4H_8-CO_2-C_2H_5)$^+$, 14.2), 72 ((M-C_4H_8-CO_2-C_2H_5.CO_2)$^+$, 79.9). (Found M$^+$: 246.1333. $C_{11}H_{20}NO_5$ requires 246.1341). ν_{max} (nujol mull)/cm^{-1} 3430, 1770, 1705.

Preparation of ethyl (*S*)-2-(*N*-*t*-butyloxycarbonyl)-amino-3-hydroxy butanoate (182)[115].

A solution of ethyl-(*S*)-(*N*-*t*-butyloxycarbonyl)-amino-3,4-epoxybutanoate (0.11g, 0.45mmol), fresh Raney nickel (1cm^3 slurry) in ethanol (50cm^3) was shaken at 100°C and 10 atmospheres of pressure for 1 hour and allowed to cool overnight. The reaction mixture was filtered through a bed of celite and washed with ethanol (10cm^3). The solution was evaporated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate / petroleum ether 3 : 1 to give

the title compound (182) as a diastereomeric mixture (0.066g, 60%). δ_H (220 MHz; $CDCl_3$) : δ_H 5.46 (1H, m, NH), 4.34 (1H, m, *CH*), 4.23 (2H, q, J 7.1 Hz, CH_2 Et), 3.86 (1H, overlapping q, J 8 Hz, *CH*OH), 1.47 (9H, s, *t*-Bu), 1.29 (6H, m, CH_3 & CH_3 Et). ν_{max} ($CHCl_3$)/cm^{-1} 3439, 3012, 1713, 1456, 1439.

Preparation of ethyl 2-(*N*-*t*-butyloxycarbonylamino)-3,4-dihydroxy butanoate (183).

A solution of ethyl (*S*)-2-(*N*-*t*-butyloxycarbonyl)-amino-3-butenoate (0.1g, 0.4mmol), 4-methylmorpholine *N*-oxide (0.1g, 0.8mmol) and osmium tetraoxide (0.1eq., 14.6µl, 0.04mmol) in *t*-butanol / water (8 : 1, $5cm^3$) was stirred for 24 hours at room temperature. Sodium sulphite (0.9g) was added to destroy the OsO_4. The mixture was partitioned between ethyl acetate ($10cm^3$) and water ($10cm^3$). The organic layer was washed with water ($10cm^3$) and saturated brine ($10cm^3$), dried over magnesium sulphate and concentrated under reduced pressure to give the crude mixture of diols which were separable by careful flash chromatography with ethyl acetate / petroleum ether 2 : 1 to give ethyl (2*S*, 3*R*)-2-(*N*-*t*-butyloxycarbonyl)amino-3,4-dihydroxybutanoate (19mg, 17%). M. p. 77-78°C. R_f = 0.23 (ethyl acetate / petroleum ether 2 : 1). δ_H (400 MHz; $CDCl_3$) 5.46 (1H, br. d, J 7.7 Hz, *NH*), 4.34 (1H, br. t, J 7.02 Hz, *CH*), 4.23 (2H, q, J 7.2 Hz, CH_2 Et), 3.86 (1H, m, *CH*OH), 3.68 (2H, m, CH_2OH), 1.43 (9H, s, *t*-Bu), 1.29 (3H, t, J 7.2 Hz, CH_3 Et). δ_C (100 MHz, solvent $CDCl_3$) 170.7 (C1), 156.3 (C=O Boc), 80.8 (C-Me_3 Boc), 73.0 (C3), 62.7 (C2), 61.9 (C4), 55.7 (CH_2 Et), 28.1 (CH_3 Boc), 14.0 (CH_3 Et). m/z (CI) 264 ($(M+1)^+$, 11%), 235 ($(M-C_2H_5)^+$, 30.6), 225 ($(M-C_4H_8+NH_3)^+$, 40.8), 208 ($(M-C_4H_8)^+$, 38.4), 188 (6.0), 179 ($(M-C_4H_8-C_2H_5)^+$, 34.6), 164 ($(M-C_4H_8-CO_2)^+$, 87.7), 144 (9), 118 (3.8), 90 ($(M-C_4H_8-CO_2-C_2H_5-CO_2)^+$, 2.8). ν_{max} ($CHCl_3$)/cm^{-1} 1792, 1713.

and an oil ethyl (2*S*, 3*S*)-2-(*N*-*t*-butyloxycarbonyl)amino-3,4-dihydroxy butanoate (45mg, 40%). R_f = 0.26 (ethyl acetate / petroleum ether 2 : 1). δ_H (400 MHz; $CDCl_3$) 5.56 (1H, br. d, J 8.2 Hz, N*H*), 4.44 (1H, dd, 8.2, 1.5 Hz, C*H*), 4.2 (3H, m+2xq, J 7.2 Hz, C*H*OH and CH_2 Et), 3.5 (2H, m, CH_2OH), 1.42 (9H, s, *t*-Bu), 1.27 (3H, t, J 7.1 Hz, CH_3 Et). δ_C (100 MHz, $CDCl_3$) 170.8 (C1), 156.8 (C=O Boc), 80.7 (C-Me_3 Boc), 72.0 (C3), 62.7 (C2), 61.9 (C4), 55.0 (CH_2 Et), 28.1 (CH_3 Boc), 14.0 (CH_3 Et). m/z (CI) 264 ((M+1)$^+$, 9.4%), 235 ((M-C_2H_5)$^+$, 6.3), 225 ((M-C_4H_8+NH_3)$^+$, 29.1), 208 ((M-C_4H_8)$^+$, 25.1), 190 ((M-$CO_2C_2H_5$)$^+$, 2.4), 179 ((M-C_4H_8-C_2H_5)$^+$, 36.2), 164 ((M-C_4H_8-CO_2)$^+$, 43.5), 147 (4.3), 118 (1.9), 90 ((M-C_4H_8-CO_2-C_2H_5.CO_2)$^+$, 3.2). m/z (EI) 147 (8%), 116 (2.9), 101 (9.3), 90 ((M-C_4H_8-CO_2-C_2H_5.CO_2)$^+$, 7.5), 74 (5.4). ν_{max} ($CHCl_3$)/cm^{-1} 1738, 1697.

The optical purities of the resolved amino acid derivatives were examined by the optical rotation of the free amino acid prepared by the usual method of acid hydrolysis and ion exchange with Dowex 50W-8X (H^+).

δ_H (400 MHz; D_2O) 4.1 (1H, m, C*H*OH), 3.7 (1H, d, 3.9Hz, C*H*), 3.67 (2H, C*H*OH). δ_C (100 MHz, $CDCl_3$) : δ_C 69.9 (C2), 70.0 (C3), 57.3 (C4).

(2*S*, 3*R*)-2-amino-3,4-dihydroxybutanoic acid : $[\alpha]_D$ = +7.0° (H_2O, 25.8°C, c=0.25).

(2*S*, 3*S*)-2-amino-3,4-dihydroxybutanoic acid : $[\alpha]_D$ = -7.3° (H_2O, 22.9°C, c=0.46).

Preparation of ethyl (2*S*)-2-(*N*-*t*-butyloxycarbonyl)aminocyclopropyl ethanoate (184).

Ethereal diazomethane (estimated 2 eq.) was added carefully to a solution ethyl (*S*)-2-(*N*-(*t*-butyloxycarbonyl)amino)-3-butenoate (0.23g, 1.0 mmol) in diethyl ether (10cm^3). Palladium (II) acetate (5mg) was added

with an immediate evolution of gas. The excess diazomethane was blown off under a stream of nitrogen and the organic layer was filtered, washed with water ($10cm^3$), saturated brine ($10cm^3$), dried over magnesium sulphate and concentrated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate / petroleum ether 1 : 10 and distillation to give the title compound (184) as an oil (0.1g, 80%). B. p. 220°C (0.6 mm Hg, bulb to bulb). δ_H (400 MHz; $CDCl_3$) 5.05, 4.8 (1H, d, J 6.8 Hz, N*H*), 4.2 (2H, m, C*H*$_2$ Et), 3.75, 3.45 (1H, br. t, J 7.8 Hz, C*H*), 1.41 (9H, s, *t*-Bu), 1.27 (3H, t, J 7.1 Hz, C*H*$_3$ Et), 1.02 (1H, m, C*H*(CH$_2$)$_2$), 0.5 (4H, m, C*H*$_2$). δ_H (100 MHz, $CDCl_3$) 172.1 (C1), 151.6 (C=O Boc), 79.7 (C-Me$_3$ Boc), 61.0 (C2), 56.5 (CH$_2$ Et), 28.2 (CH$_3$ Boc), 14.1 (CH$_3$ Et), 13.7 (C3), 2.62 (C4, 5). m/z (CI) 244 ((M+1)$^+$, 4%), 205 (9.6), 188 ((M-C$_2$H$_5$)$^+$, 25), 170 ((M-CO$_2$C$_2$H$_5$)$^+$, 4.4), 144 ((M-C$_4$H$_8$-CO$_2$)$^+$, 22.9), 109 (1.2), 84 (3.1), 70 ((M-C$_4$H$_8$-CO$_2$-C$_2$H$_5$.CO$_2$)$^+$, 15.2). m/z (EI) 170 ((M-CO$_2$C$_2$H$_5$)$^+$, 4.8%), 114 ((M-CO$_2$C$_2$H$_5$-C$_4$H$_8$)$^+$, 2.9), 84 (6.2), 70 ((M-C$_4$H$_8$-CO$_2$-C$_2$H$_5$-CO$_2$)$^+$, 51.7).

Preparation of 2-(*S*)-amino-2-cyclopropylethanoic acid (185)[122].

Ethyl (2*S*)-2-(*N*-*t*-butyloxycarbonyl)aminocyclopropylethanoate (184) (0.97g, 0.4mmol) was refluxed in 6 M HCl ($5cm^3$) for 1 hour and treated as before to give the title compound (185) (30mg, 66%). M. p. 189°C(d). $[\alpha]_D$ = +58.7° (H$_2$O, 24.7°C, c=0.23). δ_H (400 MHz; D$_2$O) 2.99 (1H, d, J 9.8 Hz, C*H*), 1.06 (1H, m, C*H*(CH$_2$)$_2$), 0.5 (3H, m, C*H*$_2$), 0.36 (1H, m, C*H*$_2$). δ_C (100 MHz, $CDCl_3$) 173.5 (C1), 60.0 (C2), 12.5 (C3), 3.60 (C4, 5). m/z (EI FAB) 115 (M$^+$·, 13.6%), 93 (100), 75 ((M-C$_3$H$_5$)$^+$, 34.6), 58 (31.9).

Preparation of ethyl 2-(*N*-*t*-butyloxycarbonyl)amino-4-hydroxy-2-butenoate (186).

A solution of ethyl (*R*)-2-(*N*-*t*-butyloxycarbonyl)-amino-3,4-epoxybutanoate (181) (0.23g, 1.0 mmol) and potassium fluoride (1.2 eq., 0.08g, 1.1mmol) in methanol (10cm^3) was stirred at room temperature for 24 hours. The solution was partitioned between ethyl acetate (20cm^3) and water (20cm^3). The organic layer was washed with water (10cm^3) and saturated brine (10cm^3), dried over magnesium sulphate and evaporated under reduced pressure to give a residue which after flash chromatography with ethyl acetate / petroleum ether 1 : 10 gave the title compound (186) as a colourless oil (0.1g, 75%). δ_H (400 MHz; $CDCl_3$) 6.64 (1H, br. t, J 7.4 Hz, C=C*H*), 6.42 (1H, br. s, N*H*), 4.24 (2H, q, J 7.1 Hz, C*H*$_2$ Et), 4.18 (2H, br. d, J 7.4 Hz, C*H*$_2$OH), 1.46 (9H, s, *t*-Bu), 1.31 (3H, t, J 7.1Hz, C*H*$_3$ Et). δ_C (100 MHz, $CDCl_3$) 164.5 (C1), 154.0 (C=O Boc), 129.4 (C2), 126.2 (C3), 81.3 (C-Me$_3$ Boc), 61.8 (C4), 58.4 (CH_2 Et), 29.6 (CH_3 Boc), 14.0 (CH_3 Et). m/z (CI) 246 ((M+1)$^+$, 10.9%), 190 ((M-C_2H_5)$^+$, 40.7), 172 (30), 146 ((M-C_4H_8-CO_2)$^+$, 7.7), 128 (85.2), 100 (1.9). m/z (EI) 126 (0.7%), 116 (0.7), 85 (1.8), 83 (2.7). ν_{max} ($CHCl_3$)/cm^{-1} 3408, 3011, 1708, 1495.

6.4 : EXPERIMENTAL DETAILS FOR CHAPTER FIVE.

Preparation of [2-$^2H_2$]-glycine (188a)[144].

The exchangable protons of diethyl acetoamidomalonate (146) were removed by dissolving it in a solution of deuterium oxide / d_1-methanol with a catalytic amount of pyridine and evaporating the resultant solution. The d_2-diethyl acetomalonate so prepared was then added to a solution of 6 M DCl / D_2O (prepared by adding thionyl chloride to D_2O) and refluxed for 30 minutes. The solution was concentrated under reduced pressure and passed down a Dowex 50W-8X (H^+) ion exchange column. The free amino acid was eluted with 4% aqueous pyridine concentrated under reduced pressure to give the title compound (188a). No signal was seen in the proton nmr in D_2O. A sample was converted to its *t*-butyloxycarbonyl derivative δ_H (250 MHz; $CDCl_3$) 1.47 (9H, s, *t*-Bu).

Preparation of (2*S*)-[2-$^2H_1$]glycine (188b)[142].

[2-$^2H_2$]Glycine (0.35g, 4.5mmol) and pyridoxal-5-phosphate (0.03g) was added to 0.3mmol potassium phosphate buffer and the pH was readjusted to pH 7.1 with 6 M NaOH (45 cm^3). Glutamine pyruvate transaminase (ex Sigma Chemical Co. Ltd., from porcine heart, suspension in ammonium sulphate solution, pH 6.0, 320u) was added and the solution was stirred for 3 days at 37°C in the dark. The solution was directly passed over a Dowex 50W-8X (H^+) ion exchange column and the (2*S*)-[2-$^2H_1$]glycine was eluted with 4% aqueous pyridine which was concentrated under reduced pressure to give the title compound (188). A sample was converted to its *t*-butyloxycarbonyl derivative δ_H (250 MHz; $CDCl_3$) 3.98 (0.8H, m, CD*H*), 1.47 (9H, s, *t*-Bu).

Preparation of methyl (*S*)-[2-$^{2}H_{1}$]bromoacetate (189).

(2*S*)-[2-$^{2}H_{1}$]glycine (0.25g, 3.3mmol) was added in small portions over 2 hours to a vigorously stirred solution of sodium nitrite (0.31g, 4.35mmol) in hydrobromic acid (2.5 M, 20cm^{3}) at 0°C. The solution was allowed to stir overnight at room temperature. The solution was exhaustively extracted with diethyl ether (6 x 15cm^{3}), dried over magnesium sulphate and concentrated under reduced pressure. The liquid residue was taken up in further diethyl ether (25cm^{3}) and treated with ethereal diazomethane (1.5 eq.) with immediate evolution of gas. The excess diazomethane was driven off under a stream of nitrogen. The ether was fractionally distilled off and the product distilled bulb-to-bulb to give the title compound (189) (0.422g, 83%). B. p. 50°C 18 mm Hg. The distillate was used immediately in the next stage of the synthesis.

Preparation of methyl *N*-(4-methoxyphenyl)glycinate (187).

An anhydrous solution of *p*-anisidine (freshly distilled, 0.246g, 2mmol), methyl bromoacetate (0.305g, 2mmol), triethylamine (0.222g, 2.2mmol) in THF (15cm^{3}) was refluxed under a nitrogen atmosphere for two hours. The solution was filtered to remove the triethylamine hydrobromide which was washed with THF (2x15cm^{3}). The filtrate was concentrated under reduced pressure. The oily residue was purified by flash chromatography with ethyl acetate / petroleum ether 1 : 8 to give the title compound (187) (0.34g, 88%). δ_{H} (250 MHz; $CDCl_{3}$) 6.71, 6.67, 6.5, 6.46 (4H, AB q, J 8.9 Hz, Ph-*H*), 3.95 (1H, br. s, N*H*), 3.76 (1H, s, CH_{2}), 3.67 (3H, s, OCH_{3}), 3.64 (3H, s, OCH_{3}). δ_{C} (100 MHz, $CDCl_{3}$) 171.8 (C1), 152.5 ((Ph), 141.1 (Ph), 114.8 (Ph), 114.3 (Ph), 55.6 (C2), 52.1 ($CO_{2}C$) 46.5 (PhO*C*). m/z (EI) 195 ((M)$^{+}$, 1.7%), 136 (9.7), 120 (1.1), 99 (1.2) (Found M^{+} : 195.0894. $C_{10}H_{13}O_{3}N$

requires 195.0895). $\nu_{max}(CHCl_3)/cm^{-1}$ 3212, 1743, 1465. Methyl *N*-(4-methoxyphenyl)-[2-$^2H_1$]-glycinate δ_H (250 MHz; $CDCl_3$) 6.83, 6.79, 6.65, 6.62 (4H, AB q, J 8.9 Hz, Ph-*H*), 3.95 (1H, br. s, CD*H*), 3.78 (3H, s, OC*H*$_3$), 3.76 (3H, s, OC*H*$_3$). m/z (EI) 196 ((M)$^+$, 19.3%), 137 (100), 121 (11.2), 100 (7.9) (Found M$^+$: 196.0951. $C_{10}H_{12}DO_3N$ requires 196.0958).

Preparation of methyl *N*-(4-methoxyphenyl)-*N'*-(*O*-acetyl-3-hydroxy-4-methylbutanoate)glycinate (195).

An anhydrous solution of methyl *N*-(4-methoxyphenyl)glycinate (0.39g, 2mmol), *O*-acetyl-3-hydroxy-4-methylbutanoic acid (0.35g, 2.2mmol), and EEDQ (1.1 eq., 0.544g, 2.2mmol) in THF (10cm^3) was allowed to stir overnight. The solution was evaporated under reduced pressure and the residue taken up in ethyl acetate (10cm^3). The organic solution was washed with hydrochloric acid (1 M, 10cm^3), sodium bicarbonate (5%, 10cm^3) and finally with saturated brine (10cm^3). The solution was dried over magnesium sulphate and evaporated under reduced pressure. The product was purified by flash chromatography eluting with ethyl acetate / petroleum ether 1 : 8 to give the title compound as an oil (195) (0.6g, 80%). δ_H (250 MHz; $CDCl_3$) 7.43 (2H, AB q, J 9.0 Hz, α-Ph-*H*), 6.91 (2H, AB q, J 9.0 Hz, β-Ph-*H*), 4.85 (1H, d, J 5.0 Hz, COC*H*OAc), 4.75, 4.68, 3.98, 3.92 (2H, AB q, J 17.1 Hz, C*H*$_2$), 3.83 (3H, s, OC*H*$_3$), 3.74 (3H, s, OC*H*$_3$), 2.10 (3H, s, COC*H*$_3$), 2.03 (1H, m, C*H*(CH$_3$)$_2$), 0.93 (3H, d, J 6.8 Hz, CHC*H*$_3$), 0.83 (3H, d, J 6.9 Hz, CHC*H*$_3$). δ_C (100 MHz, $CDCl_3$) 170.8, 169.9, 169.5, 159.2, 134.3, 129.3, 114.5, 74.9, 55.3, 52.0, 51.8, 29.5, 20.5, 19.1, 16.6. m/z (EI) 337 (M$^{+\cdot}$, 9.4%), 241 (4.5), 195 (100), 136 (67.9) (Found M$^+$: 337.1539. $C_{17}H_{23}O_6N$ requires 337.1525). ν_{max} ($CHCl_3$)/cm^{-1} 3496, 3026, 1736, 1672, 1511. Methyl *N*-(4-methoxyphenyl)-*N'*-(*O*-acetyl-3-hydroxy-4-methylbutanoate)-[2-$^2H_1$]-glycinate δ_H (400 MHz; $CDCl_3$) 7.43 (2H, AB q, J 9.0 Hz, α-Ph-*H*), 6.91

(2H, AB q, J 9.0 Hz, β-Ph-*H*), 4.85 (1H, d, J 5.0 Hz, COC*H*OAc), 4.71 (0.3H, br. s, H^{α}), 3.94 (0.3H, br. s, H^{β}), 3.83 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 2.10 (3H, s, COCH_3), 2.03 (1H, m, CH(CH$_3$)$_2$), 0.93 (3H, d, J 6.8 Hz, CHCH_3), 0.83 (3H, d, J 6.9 Hz, CHCH_3).

Preparation of methyl *N*-(4-methoxyphenyl)-*N'*-(*O*-acetyl-mandelate) glycinate (196).

As for (195) using *O*-acetylmandelic acid (0.43g, 2.2mmol) to give the title compound as an oil (196) (0.53g, 75%). δ_H (250 MHz; $CDCl_3$) 7.43 (9H, m, Ph-*H*), 5.80 (1H, s, C*H*Ph), 4.41 (1H, d, J 17.1 Hz, H^{α}), 4.01 (1H, AB q, J 17.1 Hz, H^{β}), 3.69 (3H, s, OCH_3), 3.57 (3H, s, OCH_3), 2.01 (3H, s, COCH_3). δ_C (100 MHz, $CDCl_3$) 170.4, 169.0, 168.7, 159.5, 133.43, 133.4, 129.8, 129.0, 128.7, 128.6, 128.3, 114.4, 73.5, 55.3, 51.5, 20.5. m/z (EI) 371 ($M^{+\cdot}$, 38.7%), 340 (4.0), 241 (4.1), 195 (100), 149 (18.8), 136 (45.3) (Found M^+ : 371.1373. $C_{20}H_{21}O_6N$ requires 371.1368). ν_{max} ($CHCl_3$)/cm^{-1} 3487, 3026, 1738, 1677, 1511. Methyl *N*-(4-methoxyphenyl)-*N'*-(*O*-acetyl-mandelate)-[2-$^2H_1$]-glycinate δ_H (250 MHz; $CDCl_3$) 7.43 (9H, m, Ph-*H*), 5.89 (1H, s, C*H*Ph), 4.50 (0.35H, br. s, H^{α}), 4.01 (0.35H, br. s, H^{β}), 3.81 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 2.13 (3H, s, COCH_3).

Preparation of methyl *N*-(4-methoxyphenyl)-*N'*-(2-(*N*-*t*-butyloxycarbonyl) amino-3-butenoate) glycinate (197).

As for (195) using 2-(*N*-*t*-butyloxycarbonyl)amino-3-butenoic acid (143) (0.2g, 2.2mmol) to give the title compound as an oil (197) (0.3g, 40%). δ_H (250 MHz; $CDCl_3$) 7.31 (2H, AB q, J 8.8 Hz, α-Ph-*H*), 6.93 (2H, AB q, J 9.0 Hz, β-Ph-*H*), 5.62 (1H, m, C*H*=CH$_2$), 5.43 (1H, br. d, J 7.5 Hz, N*H*), 5.14 (2H, m, CH=CH_2), 4.8 (1H, unres. t, J 7.5 Hz, C*H*), 4.57, 4.48, 4.16, 4.07 (2H, AB q,

J 17.1 Hz, CH_2), 3.82 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 1.41 (9H, s, *t*-Bu). δ_C (100 MHz, $CDCl_3$) 171.0, 169.2, 159.5, 132.7, 129.5, 129.2, 118.1, 114.8, 79.5, 55.4, 53.2, 52.1, 38.6, 29.6, 28.3. Methyl *N*-(4-methoxyphenyl)-*N'*-(2-(*N*-*t*-butyloxycarbonyl)amino-3-butenoate)-[2-$^2H_1$]-glycinate δ_H (400 MHz; $CDCl_3$) 7.24 (2H, AB q, J 8.6 Hz, α-Ph-*H*), 6.83 (2H, AB q, J 8.9 Hz, β-Ph-*H*), 5.57 (1H, ddd, J 16.8, 10.4, 6.1 Hz, CH=CH_2), 5.36 (1H, br. d, J 8.3 Hz, N*H*), 5.14 (2H, m, CH=CH_2), 4.8 (1H, unres. t, J 6.8 Hz, C*H*), 4.41 (0.3H, br. s, H^{α}), 4.01 (0.3H, br. s, H^{β}), 3.74 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 1.34 (9H, s, *t*-Bu).

Preparation of methyl *N*-(4-methoxyphenyl)-*N'*-(2-bromopropanoate) glycinate (198).

As for (195) using 2-bromopropanoic acid (0.35g, 2.2mmol) to give the title compound as an oil (198) (0.55g, 83%). δ_H (250 MHz; $CDCl_3$) 7.37 (2H, AB q, J 8.3 Hz, α-Ph-*H*), 6.91 (2H, AB q, J 9.0 Hz, β-Ph-*H*), 4.55, 4.48, 4.23, 4.16 (2H, AB q, J 17.0 Hz, CH_2), 4.32 (1H, q, J 6.7 Hz, *CH*Br), 4.19 (1H, AB q, J 17.0 Hz, H^{β}), 3.84 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 1.74 (3H, d, J 6.7 Hz, $CHCH_3$). δ_C (100 MHz, $CDCl_3$) 170.3, 169.1, 159.6, 134.1, 129.0, 114.8, 55.4, 52.1, 51.7, 38.2, 21.5. m/z (EI) 331 (M^+ $^{81}Br^+$, 14.6%), 329 (M^+ $^{79}Br^+$, 15.2), 208 (5.9), 195 (43.4), 162 (12.6), 136 (100), 120 (49.2) (Found M^+ : 329.0261. $C_{13}H_{16}O_4{}^{79}BrN$ requires 329.0345). ν_{max} ($CHCl_3$)/cm^{-1} 3026, 1752, 1669, 1511, 1219. Methyl *N*-(4-methoxyphenyl)-*N'*-(2-bromopropanoate)-[2-$^2H_1$]-glycinate. δ_H (250 MHz; $CDCl_3$) 7.37 (2H, AB q, J 8.3 Hz, α-Ph-*H*), 6.91 (2H, AB q, J 9.0 Hz, β-Ph-*H*), 4.49 (0.3H, br. s, H^{α}), 4.32 (1H, q, J 6.7 Hz, *CH*Br), 4.16 (0.3H, AB q, J 17.0 Hz, H^{β}), 3.84 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 1.74 (3H, d, J 6.7 Hz, $CHCH_3$).

Preparation of methyl *N*-(4-methoxyphenyl)-*N'*-(2-bromo-2-phenyl ethanoate) glycinate (199).

As for (195) using 2-bromopropanoic acid (0.47g, 2.2mmol) to give the title compound as an oil (199) (0.59g, 76%). δ_H (250 MHz; $CDCl_3$) 7.37 (7H, m, Ph-*H*), 6.88 (2H, unres d, J 6.6 Hz, α-MeOPh-*H*), 5.43 (1H, s, C*H*Ph), 4.48, 4.41, 4.29, 4.22 (2H, AB q, J 17.1 Hz, C*H*$_2$), 3.82 (3H, s, OC*H*$_3$), 3.70 (3H, s, OC*H*$_3$). δ_C (100 MHz, $CDCl_3$) 168.9, 168.1, 159.7, 136.2, 134.0, 129.2, 128.9, 128.7, 128.5, 114.9, 55.5, 52.1, 51.9, 45.6. m/z (EI) 393 (M+^{81}Br$^+$, 37%), 391 (M+^{79}Br$^+$, 37.6), 311 (60), 252 (44.6), 195 (72), 136 (96), 120 (67.6) (Found M$^+$: 391.0428. $C_{18}H_{18}O_4{}^{79}BrN$ requires 391.050). ν_{max} ($CHCl_3$)/cm^{-1} 3029, 1752, 1674, 1511, 1219. Methyl *N*-(4-methoxyphenyl)-*N'*-(2-bromo-2-phenylethanoate)-[2-^{2}H$_1$]-glycinate. δ_H (250 MHz; $CDCl_3$) 7.37 (7H, m, Ph-*H*), 6.88 (2H, unres d, J 6.6 Hz, α-MeOPh-*H*), 5.43 (1H, s, C*H*Ph), 4.42 (0.3H, br. s, *H*$^{\alpha}$), 4.24 (0.3H, br. s, *H*$^{\beta}$), 3.82 (3H, s, OC*H*$_3$), 3.70 (3H, s, OC*H*$_3$).

Preparation of 2-bromo-*N*-(1-phenylethyl)ethanamide (200).

As for 196 using bromoacetic acid (0.47g, 2.2mmol) to give the title compound 199 which was recrystallised from ethanol / water (0.59g, 76%). M. p. 110.5-112°C. δ_H (250 MHz; $CDCl_3$) 7.25 (5H, m, Ph-*H*), 6.71 (1H, br. s, N*H*), 5.12 (1H, m, C*H*Ph), 3.96, 3.91, 3.90, 3.85 (2H, AB q, J 13.8 Hz, C*H*$_2$), 1.54 (3H, d, J 6.9 Hz, C*H*$_3$). m/z (EI) 245 (M+^{81}Br$^+$, 15.9%), 243 (M+^{79}Br$^+$, 16.1), 241 (M+^{1}H$_2$+^{81}Br+1$^+$, 1.2%), 199 (1.5), 163 (9.9), 120 (3.3). δ_H (250 MHz; $CDCl_3$) 7.25 (5H, m, Ph-*H*), 6.61 (1H, br. s, N*H*), 5.03 (1H, m, C*H*Ph), 3.89 (0.5H, t, J 1.86 Hz, D*H*$^{\alpha}$), 3.86 (0.5H, t, J 1.86 Hz, D*H*$^{\beta}$), 1.54 (3H, d, J 6.9 Hz, C*H*$_3$). δ_C (100 MHz, $CDCl_3$) 140.1, 128.7, 127.1, 125.9, 62.1, 48.4, 21.6, 14.4. ν_{max}/cm^{-1} 3415, 1675, 1525.

Bibliography.

Chapter One.

1 : Demain, A., *Chim. Oggi.*, 1989, *7*, 9.

2 : Baldwin, J. E., *Natural Product Reports*, 1988, *5*, 129.

3 : O'Sullivan, J., Bleany, R. C., Huddleston, J. A., Abraham, E. P., *Biochem. J.*, 1979, *184*, 421.

4 : Konomi, T., Herchen, S., Baldwin, J. E., Yoshida, M., Hunt, N., Demian, A. L., *Biochem. J.*, 1979, *184*, 427.

5 : Baldwin, J. E., Killin, S. J., Pratt, A. J., Sutherland, J. D., Turner, N. J., Crabbe, M. C. J., Abraham, E. P., Willis, A. C., *J. Antibiot.*, 1987, *40*, 652.

6 : Perry, D., Abraham, E. P., Baldwin, J. E., *Biochem. J.*, 1988, *255*, 345.

7 : Pang, C-P., Chakravarti, B., Adlington, R. M., Ting, H-H., White, R. L., Jayatilake, G. S., Baldwin, J. E., Abraham, E. P, *Biochem. J.*, 1984, *222*, 789.

8 : Aplin, R. T., Baldwin, J. E., Fujishima, Y., Schofield, C. J., Green, B. N., Jarvis, S. A., *FEBS*, 1990, *264*, 215.

9 : Baldwin, J. E., Gagnon, J., Ting, H-H., *FEBS*, 1985, *188*, 253.

10 : Chen, V. J., Orville, A. M., Harpel, M. R., Frolik, C. A., Surcrus, K. K., Munck, E., Lipscomb., *J. Biol. Chem.*, 1989, *264*, 2167.

11 : White, R. L., John, E. M., Baldwin, J. E., Abraham, E. P, *Biochem. J.*, 1982, *203*, 791.

12 : Bahadur, G. A., Baldwin, J. E., Wan, T., Jung, M., Abraham, E. P., Huddleston, J. A., White, R. L., *J. Chem. Soc., Chem. Commun.*, 1984, 1225.

13 : Abraham, E. P., Adlington, R. M., Baldwin, J. E., Crimmin, M. J., Field, L. D., Jayatilake, G. S., White, R. L., *J. Chem. Soc., Chem. Commun.*, 1982, 1130.

14 : Baldwin, J. E., Johnson, B. L., Usher, J. J., Abraham, E. P., Huddleston, J. A., White, R. L., *J. Chem. Soc., Chem. Commun.*, 1980, 1271.

15 : Baldwin, J. E., Adlington, R. M., Moroney, S. M., Field, L. D., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1984, 984.

16 : Baldwin, J. E., Adlington, R. M., Bradley, M., Norris, W. J., Turner, N. J. Yoshida, A., *J. Chem. Soc., Chem. Commun.*, 1988, 1125.

17 : Baldwin, J. E., Adlington, R. M., Moroney, S. M., Field, L. D., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1984, 984.

18 : Simon, H., Palm, D., *Angew. Chem. Internat. Edit.*, 1966, *5*, 920.

19 : Baldwin, J. E., Abraham, E. P., Lovel, C. G., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1984, 902.

20 : Baldwin, J. E., Bradley, M., Adlington, R. M., Norris, W. J., Turner, N. J., *Tetrahedron*, 1991, *47*, 457.

21 : Baldwin, J. E., Adlington, R. M., Robinson, N. G., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1986, 409.

22 : Baldwin, J. E., Jung, M., Usher, J. J., Abraham, E. P., Huddleston, J. A., White, R. L., *J. Chem. Soc., Chem. Commun.*, 1981, 246.

23 : Baldwin, J. E., Chakravarti, B., Field, L. D., Murphy, J. A., Whitten, K. R., *Tetrahedron*, 1982, *38*, 2773.

24 : Aberhart, D. J., Lin, L. J., *J. Chem. Soc., Perkin Trans. 1*, 1974, 2320.

25 : Baldwin, J. E., Abraham, E. P., Adlington, R. M., Chakravarti, B., Derome, A. E., Murphy, J. A., Field, L. D., Green, N. B., Ting, H-H., Usher, J. J., *J. Chem. Soc., Chem. Commun.*, 1983, 1317.

26 : Baldwin, J. E., Abraham, E. P., Adlington, R. M., Murphy, J. A., Field, L. D., Green, N. B., Ting, H-H., Usher, J. J., *J. Chem. Soc., Chem. Commun.*, 1983, 1319.

27 : Baldwin, J. E., Adlington, R. M., Turner, N. J., Domayne-Hayman, B. P., Derome, A. E., Ting, H-H., Murphy, J. A., *J. Chem. Soc., Chem. Commun.*, 1984, 1167.

28 : Baldwin, J. E., Adlington, R. M., Domayne-Hayman, B. P., Knight, G., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1987, 1661.

29 : Baldwin, J. E., Abraham, E. P., Adlington, R. M., Bahadur, G. A., Chakravarti, B., Domayne-Hayman, B. P., Field, L. D., Green, Flitsch, S. L., Jayatilake, G. S., Spakovskis, A., Ting, H-H., Turner, N. J., White, R. L., Usher, J. J., *J. Chem. Soc., Chem. Commun.*, 1984, 1225.

30 : Baldwin, J. E., Abraham, E. P., Burge, G. L., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1985, 1808.

31 : Baldwin, J. E., Adlington, R. M., Crabbe, J. C., Knight, G. C., Nomoto, T., Schofield, C. J., *J. Chem. Soc., Chem. Commun.*, 1987, 806.

32 : Baldwin, J. E., Adlington, R. M., Crabbe, J. C., Knight, G. C., Nomoto, T., Schofield, C. J., *Tetrahedron*, 1987, *43*, 4217.

33 : Baldwin, J. E., Pratt, A. J., Maloney, M. G., *Tetrahedron*, 1987, *43*, 2565.

34 : Adlington, R. M., Aplin, R. T., Baldwin, J. E., Field, L. D., John, E. M., Abraham, E. P., White, R. L., *J. Chem. Soc., Chem. Commun.*, 1982, 137.

35 : Baldwin, J. E., Adlington, R. M., Moss, N., Robinson, N., G., *J. Chem. Soc., Chem. Commun.*, 1987, 1664

36 : Baldwin, J. E., Adlington, R. M., Moss, N., *Tetrahedron*, 1989, *45*, 2856

37 : Baldwin, J. E., Norris, W. J., Freeman, R. T., Bradley, M., Adlington, R. M., Long-Fox, S., Schofield, C. J., *J. Chem. Soc., Chem. Commun.*, 1988, 1128.

38 : Baldwin, J. E., Blackburn, J. M., Sako, M., Schofield, C. J., *J. Chem. Soc., Chem. Commun.*, 1989, 970.

39 : Baldwin, J. E., Lynch, G. P., Schofield, C. J., *J. Chem. Soc., Chem. Commun.*, 1991, 736.

40 : Baldwin, J. E., Adlington, R. M., Domayne-Hayman, B. P., Ting, H-H., Turner, N. J., *J. Chem. Soc., Chem. Commun.*, 1986, 110.

41 : Baldwin, J. E., Bradley, M., Abbott, S. D., Adlington, R. M., *J. Chem. Soc., Chem. Commun.*, 1990, 1008.

42 : Baldwin, J. E., Adlington, R. M., Basak, A., Flitsch, S. L., Petursson, S., Spakovskis, A., Turner, N. J., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1986, 975.

43 : Baldwin, J. E., Adlington, R. M., Basak, A., Imming, P., Ponnamperuma, K., Ronneberg, H., Schofield, C. J., Ting, H-H., Turner, N. J., White, R. L., Usher, J. J., *J. Chem. Soc., Chem. Commun.*, 1989, 802.

44 : Baldwin, J. E., Adlington, R. M., Basak, A., Flitsch, S. L., Petursson, S., Turner, N. J., Ting, H-H., Turner, *J. Chem. Soc., Chem. Commun.*, 1986, 1305.

45 : Baldwin, J. E., Abraham, E. P., Adlington, R. M., Bahadur, G. A., Chakravarti, B., Domayne-Hayman, B. P., Field, L. D., Green, Flitsch, S. L., Jayatilake, G. S., Spakovskis, A., Ting, H-H., Turner, N. J., White, R. L., Usher, J. J., *J. Chem. Soc., Chem. Commun.*, 1986, 273.

46 : Baldwin, J. E., Adlington, R. M., Schofield, C. J., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1989, 758.

47 : Baldwin, J. E., Adlington, R. M., Basak, A., Ting, H-H., *J. Chem. Soc., Chem. Commun.*, 1986, 1280.

48 : Baldwin, J. E., Adlington, R. M., Flitsch, S. L., Ting, H-H., Turner, N. J., *J. Chem. Soc., Chem. Commun.*, 1986, 1305.

49 : Baldwin, J. E., Adlington, R. M., Bradley, M., Turner, N. J., Pitt, A. R., *J. Chem. Soc., Chem. Commun.*, 1990, 1008.

Chapter Two.

50 : Dardenne, G., Casimir, J., Larsen, P. O., *Phytochemistry*, 1974, *13*, 1897.

51 : Cho, C., Sugimoto, Y., Kim, J. M., Usuda, H., Ishii, R., Hyeon, S. B., Suzuki, A., *Agric. Biol. Chem.*, 1983, *47*, 2685.

52 : Suzuki, A., Hyeon, S. B., Nagai, I., Iesaka, H., Kajita, T., Furushima, M., Eur. Pat. Appl. EP 181,494, 1984.

53 : Matsumoto, N., *Toho Igakki Zasshi*, 1984, *31*, 249.

54 : Rando, R. R., *Biochemistry*, 1974, *13*, 3859.

55 : Lacoste, A. M., Darriet, M., Neuzil, E., LeGoffic, F., *Biochem. Soc. Trans.*, 1988, *16*, 606.

56 : Cho, C., Ishii, R., Hyeon, S. B., Suzuki, A., Suzuki, A., *Agric. Biol. Chem.*, 1987, *51*, 2597.

57 : Lai, J. C. K., Cooper, A. J. L., *J. Neurochem.*, 1986, *47*, 1376.

58 : Rando, R. R., *Science*, 1974, *185*, 320.

59 : Stryer, L., Biochemistry, 2 Ed, Freeman & Co., San Francisco.

60 : Rando, R. R., "Enzyme-Activated Irreversible Inhibitors", (Ed. Seiler, N., Jung, M. J., Koch-Weser, J.), Elsevier, 1978, 13,

61 : Friis, P., Helboe, P., Larsen, P. O., *Acta. Chem. Scand.*, 1974, *28*, 317.

62 : Sawada, S., Nakayama, T., Esaki, N., Tnanaka, H., Soda, K., Hill, R. K., *J. Org. Chem.*, 1986, *51*, 3384.

63 : Greenlee, W. J., *J. Org. Chem.*, 1984, *49*, 2632.

64 : Afzali-Ardakani, A., Rapaport, H., *J. Org. Chem.*, 1980, *45*, 4817.

65 : Meffre, P., Liliane, V-Q., Yen, V-Q., Le Goffic, F., *Synthetic Commun.*, 1989, *19*, 3457.

66 : Belokon, Y., Sagyan, A., S., Djamgaryan, S., A., Bakmutov, V., I., Vitt, S., V., Batsanov, A., S., Struchkov, Y., T., Belikov, V., M., *J. Chem. Soc., Perkin. Tran. 1*, 1990, 2301.

67 : Hanessian, S., Sahoo, S. P., *Tetrahedron. Lett.*, 1984, *25*, 1425.

68 : Barton, D. H. R., Crich, D., Herve, Y., Potier, P., Thierry, J., *Tetrahedron*, 1985, *41*, 4347.

69 : Pellicciari, R., Natalini, B., Marinozzi, M., *Syn. Commun.*, 1988, *18*, 1715.

70 : Schollkopf, U., Nozulak, J. Groth, U., *Tetrahedron*, 1984, *40*, 1409.

71 : Schollkopf, U., *Tetrahedron*, 1983, *39*, 2085.

72 : Shea, R. G., Fitzner, J. N., Frankhauser, J. E., Spaltenstein, A., Carpino, P. A., Peevey, R. M., Pratt, D. V., Tenge, B. J., Hopkins, P. B., *J. Org. Chem.*, 1986, *51*, 5243.

73 : Munster, P., Steglich, W., *Synthesis*, 1987, 223.

74 : Castelhano, A. L., Horne, S., Billedeau, R., Krantz, A., *Tetrahedron Lett.*, 1986, 27, 2435.

75 : Castelhano, A. L., Horne, S., Taylor, G. J., Billedeau, R., Krantz, A., *Tetrahedron*, 1988, *44*, 5451.

76 : Vyas, D., M., Chiang, Y., Doyle, T., W., *J. Org. Chem.*, 1984, *49*, 2037.

77 : Beaulieu, P., L., Duceppe, J-S., Johnson, C., *J. Org. Chem.*, 1991, *56*, 4196.

78 : Mulzer, J., Angermann, B., S., Sielz, C., *J. Org. Chem.*, 1986, *51*, 5294.

79 : Neber, F. W., von Friedolsheim, A., *Ann.* 1926, *449*, 109.

80 : Neber, F. W., Huh, U., *Ann.* 1935, *515*, 283.

81 : Neber, F. W., Burgard, A., Their, W., *Ann.* 1936, *526*, 277.

82 : Neber, F. W., Burgard, A., *Ann.* 1932, *493*, 281.

83 : Cram, D. J., Hatch, M. S., *J. Am. Chem.*, 1953, *75*, 33.

84 : Cram, D. J., Hatch, M. S., *J. Am. Chem.*, 1953, *75*, 38.

85 : House, H. O., Berkowitz, W. F., *J. Org. Chem.*, 1963, *28*, 2271.

86 : House, H. O., Berkowitz, W. F., *J. Org. Chem.*, 1963, *28*, 307.

87 : Smolinsky, G., *J. Org. Chem.*, 1962, *27*, 3557.

88 : Delgado, A., Garcia, J. M., Mauleon, D., Minguillon, C., Subirats, J. R., Feliz, M., Lopez, F., Velasco, D., *Can. J. Chem.*, 1988, *66*, 517.

89 : Zemlicka, J., Murata, M., *J. Org. Chem.*, 1976, *41*, 3317.

90 : LaMattina, J. L., *J. Heterocycl. Chem.*, 1983, *20*, 533.

Chapter Three.

91 : Meienhofer, J., Kuromizu, K., *Tetrahedron Lett.*, 1974, *37*, 3259.

92 : Claesen, M., Vlietnick, A., Vanderhaeghe, H., *Bull. Soc. Chim. Belges.*, 1968, *77*, 587.

92 : Baldwin, J. E., Killin, S. J., Adlington, R. A., Spiegel, U., *Tetrahedron*, 1988, *44*, 2633.

94 : Baldwin, J. E., Harrison, P., Murphy, J. A., *J. Chem. Soc., Chem. Commun.*, 1982, 818.

95 : Gamcsik, M. P., Malthouse, J. P. G., Primrose, W. U., Mackenzie, N. E., Boyd, A. S. F., Russel, R. A., Scott, A. I., *J. Am. Chem.*, 1983, *105*, 6324.

96 : Cantacuzene, D., Pascal, F., Guerreiro, C., *Tetrahedron*, 1987, *43*, 1823.

97 : Semenov, A. N., Khmelnitski, Y. L., Berezin, I. V., Martinek, K., *Biocatalysis*, 1987, *1*, 3.

98 : Baldwin, J. E., Hershen, S. J., Johnson, B. L., Jung, M., Usher, J. J., Wan, T., *J. Chem. Soc., Perkin Trans. 1*, 1981, 2253.

99 : Moroder, L., Hallett, A., Wunch, E., Keller, O., Wersin, G., *Hoppe-Seyler's Z. Physiol. Chem.*, 1976, *357*, 1651.

100 : Yates, P., Shapiro, B. L., *J. Org. Chem.*, 1958, *23*, 759.

101 : Goodacre, J., Ponsford, R. J., Stirling, I., *Tetrahedron Lett.*, 1975, *42*, 3609.

102 : Simkins, N. S., *Tetrahedron*, 1990, *46*, 6951.

103 : Julia, M., Lauron, H., Stacino, J-P., Verpeaux, J-N., *Tetrahedron*, 1986, *42*, 2475.

104 : Julia, M., Stacino, J-P., *Bull. Soc. Chim. France*, 1985, 831.

Chapter Four.

105 : Sigma Catalogue, 1990, 1033.

106 : Friis, P., Olsen, C. E., *J. Chem. Soc., Perkin Trans. 1*, 1977, 661.

107 : Crout, D. H. G., Lutsdorf, M., Morgan, P. J., *Tetrahedron*, 1983, *39*, 3457.

108 : Lee, Y-N., Schmir, G. L., *J. Am. Chem. Soc.*, 1978, *100*, 6700.

109 : Lee, Y-N., Schmir, G. L., *J. Am. Chem. Soc.*, 1979, *101*, 3026.

110 : Graham, W. *Tetrahedron Lett.*, 1969, *20*, 2223.

111 : Tamele, M., Ott, C. J., Marple, K. E., Hearne, G., *Ind. Eng. Chem.*, 1941, *33*, 115.

112 : Emziane, M., Lhoste, P., Sinon, D., *Synthesis*, 1988, 541.

113 : Hirsch, J. A., Truc, V. C., *J. Org. Chem.*, 1986, *51*, 2218.

114 : Dryak, V. G., *Tetrahedron*, 1976, *32*, 2855.

115 : Mitsui, S., Sugi, Y., Yokoo, K., *Tetrahedron*, 1974, *30*, 31.

116 : Meffre, P., Vo-Quang, L., Vo-quang, Y., Le Goffic, F., *Tetrahedron Lett.*, 1990, *31*, 2291.

117 : Mitchell, R. E., *Phytochem.*, 1989, *28*, 1617.

118 : Cha, J. H., Christ, J. K., Kishi, Y., *Tetrahedron Lett.*, 1983, *24*, 3943.

119 : Van Rheenan, V., Kelly, R. C., Cha, D. Y., *Tetrahedron Lett.*, 1976, *23*, 1773.

120 : Rawson, R. J., Harrison, I. T., *J. Org. Chem.*, 1970, *35*, 2057.

121 : Repic, O., Vogt, S., *Tetrahedron Lett.*, 1982, *23*, 2729.

122 : Suda, M., *synthesis.*, 1981, 714.

122 : Anciaux, A. J., Hubert, A. J., Noels, A. F., Petiniot, N., Teyssie., *J. Org. Chem.*, 1980, *45*, 695.

123 : Anderson, J. W., Fowden, L., *Biochem. J.*, 1970, *119*, 691.

124 : Olah, G. A., Welch, J. T., Vankar, Y. A., Nojima, M., Kerekes, I., Olah, J., *J. Org. Chem.*, 1979, *44*, 3872.

Chapter Five.

125 : Cacchi, S., Gargani, G., Gaspaani, F., *Chim. Oggi.*, 1984, *12*. 11.

126 : Schuring, V., Nowoty, H., P., Angew. *Chem. Int. Engl.*, 1990, 29, 939.

127 : Pirkle, W. H., Sikkenga, D. L., *J. Org. Chem.*, 1977, *42*, 1370.

128 : Pirkle, W. H., Hoover, D. J., *Top. stereochem.*, 1982, *13*, 263.

129 : Sullivan, G. R., *Top. stereochem.*, 1977, *10*, 287.

130 : Parker, D., *J. Chem. Soc., Perkins Trans. 1*, ., 1983, 83.

131 : Dale, J. A., Dull, D. L., Mosher, H. S., *J. Org. Chem.*, 1969, *42*, 2543.

132 : Hamon, D. P. G., Razzino, P., Massy-Westropp, R. A., *Tetrahedron Lett.*, 1988, *44*, 5507.

133 : Ramalingam, K., Palaniappagownder, N., Kalvin, D. M., Woodward, R.W., *Tetrahedron*, 1988, *44*, 5597.

134 : Santaniello, E., Casati, R., Manzocchi, A., *J. Chem. Soc., Perkin. Trans. 1*, 1985, 2389.

135 : Ohrui, H., Misawa, T., Meguro, H., *J. Org. Chem*, 1985, *50*, 3007.

136 : Belokon, Y. N., Melikyan, A. S., Salel'eva, T. F., Bakhutov, V. I., Vitt, S. V., Belikov, V. M., *Tetrahedron*, 1980, *36*, 2327.

137 : Yamada, H., O'Leary, M. H., *Biochemistry*, 1978, *17*, 672.

138 : Armarego, W. L. F., Milloy, B. A., Pendergast, W., *J. Chem. Soc., Perkins Trans. 1*, 1976, 2229.

139 : Fuganti, C., Mazza, M., *J. Chem. Soc., Perkins Trans. 1*, 1973, 954.

140 : Gani, D., Wallis, O. C., Young, D. W., *Eur. J. Biochem.*, 1983, *136*, 303.

141 : Besmer, P., Arigoni, D., *Chemia*, 1968, *22*, 190.

142 : Besmer, P., Ph.D. Thesis, University of Zurich, 1969.

143 : Upson, D. A., Hruby, V. J., *Chemia*, 1968, *22*, 494.

144 : Blomquist, A. T., Hiscock, B. F., Harpp, D. N., *J. Org. Chem.*, 1968, *22*, 494.

145 : Faustini, F., De Munari, S., Panzeri, A., Villa, V., Gandolfi, C. A., *Tetrahedron Lett.*, 1982, *22*, 4533.

146 : Beyerman, H. C., Maat, L., Noordam, A., van Zon, A., *Recl. Trav. Chim. Pays-Bas*, 1977, *96*, 222.

147 : Beyerman, H. C., Buijen van Weelderen, A. W., Maat, L., Noordam, A., *Recl. Trav. Chim. Pays-Bas*, 1977, *96*, 190.

TABLE 1. Atom coordinates (x10⁴) and isotropic thermal parameters (Å²x10

Atom	x	y	z	U
O(1)	1155(9)	428(5)	207(2)	57(2)*
O(2)	2408(9)	-371(5)	1632(2)	50(2)*
O(3)	-2982(12)	-3534(6)	610(3)	69(2)*
O(4)	-3444(10)	-1068(6)	36(2)	66(2)*
O(5)	-909(9)	1620(5)	831(2)	52(2)*
O(6)	-492(8)	127(4)	2271(2)	41(1)*
N(1)	-1657(11)	-541(6)	1452(2)	39(2)*
C(1)	-215(14)	479(7)	602(3)	43(2)*
C(2)	-1298(12)	-739(6)	866(2)	35(2)*
C(3)	-3754(14)	-1136(8)	604(3)	46(2)*
C(4)	-4590(15)	-2516(8)	791(3)	57(3)*
C(5)	291(14)	-255(7)	1778(3)	37(2)*
C(6)	1231(12)	521(7)	2703(3)	37(2)*
C(7)	2729(15)	-694(7)	2887(3)	57(3)*
C(8)	-487(14)	966(8)	3156(3)	50(3)*
C(9)	2905(14)	1651(7)	2519(3)	51(3)*
C(10)	206(17)	2859(8)	623(3)	66(3)*
C(11)	2645(19)	3077(8)	883(3)	69(3)*

* Equivalent isotropic U defined as one third of the trace of the orthogonalised U_{ij} tensor

TABLE 2. Bond lengths (A)

O(1)-C(1)	1.221(9)	O(2)-C(5)	1.210(9)
O(3)-C(4)	1.409(10)	O(4)-C(3)	1.400(8)
O(5)-C(1)	1.320(9)	O(5)-C(10)	1.465(10)
O(6)-C(5)	1.331(8)	O(6)-C(6)	1.465(8)
N(1)-C(2)	1.460(8)	N(1)-C(5)	1.355(10)
C(1)-C(2)	1.493(10)	C(2)-C(3)	1.531(10)
C(3)-C(4)	1.516(11)	C(6)-C(7)	1.525(10)
C(6)-C(8)	1.514(10)	C(6)-C(9)	1.514(10)
C(10)-C(11)	1.485(14)		

TABLE 3. Bond angles (deg.)

C(1)-O(5)-C(10)	117.3(6)	C(5)-O(6)-C(6)	121.6(5)
C(2)-N(1)-C(5)	120.1(6)	O(1)-C(1)-O(5)	123.0(7)
O(1)-C(1)-C(2)	123.2(7)	O(5)-C(1)-C(2)	113.7(6)
N(1)-C(2)-C(1)	111.5(5)	N(1)-C(2)-C(3)	109.2(5)
C(1)-C(2)-C(3)	111.9(6)	O(4)-C(3)-C(2)	107.2(6)
O(4)-C(3)-C(4)	112.3(6)	C(2)-C(3)-C(4)	111.6(6)
O(3)-C(4)-C(3)	111.8(6)	O(2)-C(5)-O(6)	126.7(6)
O(2)-C(5)-N(1)	123.3(6)	O(6)-C(5)-N(1)	110.0(6)
O(6)-C(6)-C(7)	109.9(5)	O(6)-C(6)-C(8)	102.3(5)
C(7)-C(6)-C(8)	110.2(6)	O(6)-C(6)-C(9)	111.6(5)
C(7)-C(6)-C(9)	110.8(6)	C(8)-C(6)-C(9)	111.7(6)
O(5)-C(10)-C(11)	110.1(6)		

TABLE 4. Anisotropic thermal parameters ($A^2 \times 10^3$)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	60(3)	59(3)	54(3)	-7(3)	10(3)	-12(3)
O(2)	34(3)	67(4)	48(3)	-16(3)	3(2)	-4(3)
O(3)	67(4)	55(3)	86(4)	-26(3)	-15(4)	1(4)
O(4)	54(3)	96(4)	49(3)	-16(3)	-3(3)	-6(4)
O(5)	52(3)	42(3)	62(3)	-2(3)	12(3)	4(3)
O(6)	32(2)	52(3)	40(2)	-11(2)	2(2)	-0(2)
N(1)	30(4)	51(4)	35(3)	-10(3)	1(3)	2(4)
C(1)	40(4)	46(5)	42(4)	-6(4)	3(4)	6(4)
C(2)	28(4)	41(4)	35(4)	-9(3)	2(4)	-3(4)
C(3)	41(4)	56(5)	41(4)	-11(4)	-2(4)	-8(4)
C(4)	41(5)	67(6)	67(5)	-19(5)	0(4)	-10(5)
C(5)	42(4)	37(4)	33(4)	-3(3)	-0(4)	-12(4)
C(6)	31(4)	41(4)	39(3)	-6(3)	-11(4)	-5(4)
C(7)	47(5)	56(5)	67(5)	2(4)	-10(4)	2(5)
C(8)	47(4)	65(5)	38(4)	-7(4)	-1(4)	-14(5)
C(9)	46(5)	46(5)	60(5)	-13(4)	-3(4)	-6(5)
C(10)	71(7)	57(5)	71(5)	1(5)	6(6)	1(5)
C(11)	85(7)	51(5)	73(6)	1(5)	9(6)	-16(5)

The anisotropic thermal parameter exponent takes the form:

$-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + \ldots + 2hka^*b^*U_{12})$

TABLE 5. H-atom coordinates ($x10^4$) and thermal parameters (A^2x10^3)

Atom	x	y	z	U
H(2)	-138	-1446	797	80
H(3)	-5044	-550	728	80
H(4a)	-6196	-2684	642	80
H(4b)	-4671	-2535	1183	80
H(7a)	3685(15)	-485(7)	3206(3)	80
H(7b)	3796(15)	-1019(7)	2603(3)	80
H(7c)	1537(15)	-1372(7)	2976(3)	80
H(8a)	430(14)	1227(8)	3475(3)	80
H(8b)	-1755(14)	343(8)	3263(3)	80
H(8c)	-1221(14)	1746(8)	2993(3)	80
H(9a)	3760(14)	2123(7)	2804(3)	80
H(9b)	1942(14)	2276(7)	2310(3)	80
H(9c)	4078(14)	1218(7)	2284(3)	80
H(10a)	352	2822	232	80
H(10b)	-833	3600	724	80
H(11a)	3383(19)	3936(8)	811(3)	80
H(11b)	3719(19)	2377(8)	755(3)	80
H(11c)	2390(19)	2977(8)	1270(3)	80
H(1)	-2946(105)	-265(58)	1561(21)	11(17)
H(31)	-3885(227)	-4153(108)	525(46)	158(57)
H(4)	-5181(209)	-862(102)	-141(37)	128(36)